Catecholamines and arrhythmias in the anaesthetized rat

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CATECHOLAMINES AND ARRHYTHMIAS IN THE ANAESTHETIZED RAT

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SUMMARY

This study initially investigated the effects of the neuronal uptake blocking agent desipramine and the α_2-adrenoceptor antagonists yohimbine and idazoxan, both alone and in combination, on haemodynamic parameters and coronary artery occlusion-induced arrhythmias in the anaesthetized rat. Desipramine had a dose related antiarrhythmic effect which has been attributed to its reported ability to inhibit membrane conductances to sodium and calcium ions. Antagonism of presynaptic α_2-adrenoceptors exacerbated ischaemia-induced arrhythmias, although high doses of yohimbine could provide protection by virtue of local anaesthetic effects. Paradoxically, concomitant administration of desipramine, at a dose that did not affect arrhythmias on its own but appeared to inhibit neuronal uptake, abolished the arrhythmogenic effects of α_2-adrenoceptor antagonism. Desipramine did not affect the antiarrhythmic action of the high dose of yohimbine and bilateral vagotomy was without effect on arrhythmias in animals treated with this combination.

Measurement of plasma catecholamine levels after coronary occlusion, prior to the development of arrhythmias, revealed no direct relationship between the effects of drugs on these levels and their effects on arrhythmias. There was no evidence of an important arrhythmogenic role for circulating catecholamines following coronary occlusion.

Studies with potassium-selective electrodes in non-ischaemic anaesthetized rats suggested that drug interventions elevating plasma catecholamine levels tended to reduce plasma potassium concentration by a β-adrenoceptor mediated mechanism. Further studies are required to determine whether such changes are significant in open-chest animals in the setting of myocardial ischaemia and whether they affect the severity of arrhythmias.

CONTENTS

Chapter 1

INTRODUCTION

1.1. Ischaemia-induced arrhythmias: clinical relevance

Cardiovascular diseases are the most common cause of mortality in the western world, accounting for approximately 50% of all deaths in people over 20 years of age, of which 70% are attributable to ischaemic heart disease (Pantridge, Adgey, Geddes and Webb, 1975). The clinical conditions of myocardial ischaemia and infarction lead to over 100,000 hospital admissions per year in Britain (Greenbaum, 1985). A large proportion of deaths from these conditions, however, are sudden and occur before the patient has reached hospital. The Edinburgh Community Study showed that, of all deaths occurring within 4 weeks of the onset of a heart atack, 50% took place within the first 2 hours (Armstrong, Duncan, Oliver, Julian, Donald, Fulton, Lutz and Morrison, 1972).

One of the early consequences of coronary occlusion is the development of arrhythmias that may culminate in ventricular fibrillation, which is considered to be the major cause of sudden cardiac death (Oliver, 1982). The delineation of the basic cellular, biochemical and electrophysiological mechanisms responsible for these potentially fatal arrhythmias is, therefore, of utmost importance, with a view to developing effective treatments.

1.2. Experimental models of myocardial ischaemia

In man, myocardial ischaemia may result from coronary thrombosis, vasospasm or an increased workload in the presence of a fixed obstruction, such as an atheromatous plaque, and is defined as an imbalance between the myocardial demand for and the vascular supply of coronary blood. In experimental animals, there are a variety of techniques by which blood flow to the coronary vasculature

can be restricted, resulting in myocardial ischaemia.

The most commonly employed method involves occlusion of a coronary artery (usually the left anterior descending or circumflex) by tightening of a previously placed ligature, usually in anaesthetized animals (Kelliher, Dix and Soifer, 1983). The acute occlusion technique has been frequently used in the dog to evaluate the biochemical, haemodynamic and electrical consequences of ischaemia since the early studies of Harris (1950). Many studies have been reported using the cat, pig, rabbit, rat and even the baboon (for references see Botting, Curtis and Walker, 1986; Kelliher et al, 1983). Isolated hearts from rats (Daugherty and Woodward, 1982; Abrahamsson, Almgren and Carlsson, 1983) and guinea pigs (Penny, 1984) have also been frequently used to study the consequences of ischaemia. In the latter species, however, regional myocardial ischaemia is difficult to induce by coronary occlusion because of the highly developed collateral circulation (Schaper, 1984). Therefore, global ischaemia produced by flow reduction or cessation is used.

In general, there is a high degree of similarity between the arrhythmias seen at various times after the onset of experimentally induced ischaemia and those observed clinically (Botting et al, 1986). The first phase of arrhythmias start within a few minutes of acute coronary occlusion, increase in frequency (sometimes precipitating into ventricular fibrillation) and revert to a sinus rhythm by about 30 min. This is commonly referred to as the early phase of arrhythmias and corresponds in humans to the potentially fatal period immediately after the onset of symptoms, before patients

reach the hospital. In some experimental models these early ventricular arrhythmias can be further divided into two discrete phases (1a and 1b) which may arise through different electrophysiological mechanisms (Russell, Lawrie, Riemersma and Oliver, 1984).

A later phase of arrhythmias begin approximately 4-8 h after coronary artery occlusion and may persist for 2-3 days. These arrhythmias are usually less severe than those seen in the early phase and correspond to those observed in humans during the period after occlusion when the patient would be in the coronary care unit (Kelliher et al, 1983).

1.3. Metabolic and ionic changes during myocardial ischaemia

During severe ischaemia, a well recognised series of biochemical and physiological changes occur before cell necrosis (infarction). These changes are highly heterogeneous, with temporal and regional differences, and may predispose to arrhythmias (Hearse and Dennis, 1982).

Following the onset of ischaemia, available oxygen is utilized within the first few seconds leading to a major reduction of oxidative metabolism, which in turn causes the depletion of high energy phosphates. Subsequently, anaerobic glycolysis is stimulated resulting in the accumulation of lactate and H^+ (Hearse and Dennis, 1982). Accompanying the rise in H^+ concentration is an increase in extracellular K^+ concentration (Hirche, Friedrich, Kebbel, McDonald and Zylka, 1982; Hill and Gettes, 1980). Catecholamines may be released (Riemersma, 1982) elevating tissue cAMP levels (Podzuweit, 1982). Free fatty acid levels are elevated and this may lead to the

accumulation of long chain acylcarnitines in the ischaemic area, in the presence of depressed β-oxidation (Corr and Sobel, 1982). Lysophospholipids also accumulate in the ischaemic myocardium (Corr and Sobel, 1979; 1982; 1983). Arachidonic acid metabolites are released into blood draining from the ischaemic myocardium (Coker, 1982). Enhanced metabolism of arachidonic acid and other changes, such as the conversion of xanthine dehydrogenase to xanthine oxidase and the accumulation of hypoxanthine, may predispose the ischaemic tissue to the production of highly reactive free radicals if oxygen is reintroduced (Werns, Shea and Lucchesi, 1986). With increasing duration of ischaemia intracellular accumulation of both sodium and calcium takes place, followed by a profound disruption of the ischaemic cells.

Many of these changes have been implicated in the genesis of ischaemia-induced arrhythmias and their possible roles will be discussed later in this chapter.

1.4. Mechanism of ischaemia-induced arrhythmias

The major mechanism of arrhythmogenesis in the early phase is widely believed to be re-entry whereas late phase arrhythmias may depend primarily on enhanced automaticity (Corr and Sobel, 1979). Late phase arrhythmias are outside the scope of this study and will not be discussed in detail.

The prerequisite for re-entry is unidirectional block of impulse conduction which will allow retrograde "re-excitation" of tissue proximal to the area of conduction block. However, certain other conditions must also be present to allow such re-excitation to occur.

These are slowed conduction velocity and/or shortened refractoriness, which allow the tissue proximal to the area of unidirectional block to recover excitability. These conditions have indeed been observed during acute myocardial ischaemia and re-entry circuits have been demonstrated (Russell, 1982; Janse, 1982; Janse, Kleber, Capucci, Coronel and Wilms-Schopman, 1986). It has been suggested, however, that the early period of arhythmias can be further divided into two electrophysiologically distinct phases (1a and 1b) with re-entry far more likely during phase 1a (Russell et al, 1984).

The most important factor responsible for the development of conditions favourable to re-entry during acute ischaemia is probably the extracellular accumulation of potassium. Indeed, Weiss and Shine (1982) have shown that hyperkalaemia equivalent to that occurring during ischaemia, in combination with acidosis and catecholamines, reproduced the electrophysiological changes observed during ischaemia such as shortened action potential duration and increased conduction time in the isolated rabbit interventricular septum. The extracellular accumulation of potassium causes a reduction in resting membrane potential which inactivates the fast sodium current and can give rise to slow-response action potentials characterized by depolarisation dependent on a slow inward current carried by either calcium or sodium (Corr and Sobel, 1979). These changes in impulse conduction within the ischaemic area are inhomogeneous and may result in adjacent areas of slow conduction and conduction block - a combination highly favourable to re-entry (Wit, 1985).

1.5. Myocardial catecholamine metabolism

The heart is heavily innervated by sympathetic fibres arising

from cervical, thoracic and stellate ganglia. The right atrium and sinoatrial node are mainly innervated by the right stellate cardiac nerve whereas most of the remaining innervation is by axons from the left sympathetic nerves (Manger, 1982).

The main catecholamine in post-ganglionic sympathetic nerve fibres is noradrenaline. Noradrenaline is released by the nerve terminals upon stimulation by tne calcium-dependent process of exocytosis whereby storage vesicles expel their contents into the extracellular fluid. Majority (80-90%) of the noradrenaline released into the synaptic cleft is removed by the neuronal reuptake process which has been described by Iversen (1967). There is also some extraneuronal uptake and the remaining noradrenaline overflows into the circulation. Any free noradrenaline in the cytoplasm of sympathetic nerves is deaminated by monoamine oxidase (MAO) whereas extraneuronal catabolism is mainly by catechol-o-methyltransferase (COMT).

The release of noradrenaline by sympathetic nerve terminals may be regulated by presynaptic adrenoceptors (Langer, 1979). Stimulation of presynaptic α_2-adrenoceptors inhibits noradrenaline release whereas their blockade by selective α_2-adrenoceptor antagonists has been shown to enhance noradrenaline release during nerve stimulation (Yamaguchi, DeChamplain and Nadeau, 1977; Dart, Dietz, Hieronymus, Kubler, Mayer, Schomig and Strasser, 1984). A positive feedback mechanism mediated by presynaptic β-adrenoceptors has also been suggested but its physiological significance is not clear. Other presynaptic receptors, such as acetylcholine, prostaglandin and adenosine receptors, may also modulate

noradrenaline release by sympathetic nerve terminals (Fuder, 1985). The main features of noradrenaline release and removal are summarised in figure 1.

Although measurement of arterial catecholamine levels cannot differentiate between release from the heart and other organs, plasma levels of noradrenaline correlate well with sympathetic nerve activity and the plasma adrenaline concentration is a good indicator of adrenomedullary activity (Manger, 1982).

1.6. Catecholamine release during myocardial ischaemia

There is a considerable body of evidence suggesting that an increase in sympathetic activity and catecholamine release occurs during myocardial ischaemia and infarction in both the clinical and experimental situation, although the experimental evidence is somewhat contradictory.

Pantridge, Webb and Adgey (1981) reported that over one third of 89 patients, seen within 30 min of the onset of acute myocardial infarction, showed evidence of sympathetic overactivity. Urine (Jewitt , Mercer, Reid, Valori, Thomas and Shillingford, 1969) and plasma (Sorkin, Tokarsky, Huber-Smith, Steiger, McCann, Arbor and Arbor, 1982) concentrations of catecholamines have been found to increase in patients with acute myocardial infarction. Suggesting a relationship between plasma catecholamine levels and arrhythmogenesis, the highest plasma levels of noradrenaline and adrenaline were found in patients with ventricular fibrillation, followed by myocardial infarction without ventricular fibrillation, and then by chest pain without infarction (Bertel, Buhler, Baitsch, Ritz and Burkart, 1982). However such findings are not conclusive as

the elevated plasma catecholamine levels could have been a consequence rather than the cause of the arrhythmias.

Several experimental findings also indicate an increased adrenergic activity following acute myocardial ischaemia. Both afferent (Uchida and Murao, 1974) and efferent cardiac sympathetic nerve activity have been shown to increase within minutes of coronary occlusion, the latter occurring either as part of a general sympathetic activation (Karlsberg, Penkoske, Cryer, Corr and Roberts, 1979) or caused by a local cardiac reflex (Brown and Malliani, 1971). A reduced catecholamine fluorescence within the ischaemic myocardium has been demonstrated 30 min and 1 h after coronary artery occlusion respectively in the anaesthetized rat (Holmgren, Abrahamsson, Almgren and Eriksson, 1981) and dog (Muntz, Hagler, Boulas, Willerson and Buja, 1984). Abrahamsson, Almgren and Svensson (1981) also showed a significant reduction in the noradrenaline content of the ischaemic zone within 20 min of coronary occlusion. Results from experiments using the ganglion blocker chlorisondamine led these workers to speculate that although the early loss of myocardial noradrenaline (within 30 min of ischaemia) might be due to a combination of local ischaemia induced release and increased sympathetic nerve activation of the heart, later reductions in noradrenaline levels (after 2.5 h of ischaemia) were almost entirely due to a local, nerve impulse independent release process (Abrahamsson, Almgren and Holmgren, 1982).

Increased overflow of noradrenaline during acute myocardial ischaemia has been demonstrated with the isolated Langendorff-perfused rat heart, using both regional ischaemia by coronary

occlusion (Abrahamsson, Almgren and Carlsson, 1983) and global ischaemia by reduction or cessation of flow (Schomig, Dietz, Strasser, Dart and Kubler, 1982), the former study using hearts labelled with tritiated noradrenaline. In both of these studies the coronary effluent was collected during reperfusion and the increased efflux of noradrenaline mighthave been caused by the reperfusion process per se. However, the outflow of noradrenaline on reperfusion after 60 min of regional ischaemia was not diminished when calcium, the prime mediator of reperfusion damage, was omitted from the reperfusion medium (Abrahamsson, Almgren and Carlsson, 1984). Calcium was probably involved in noradrenaline release during ischaemia as pre-perfusion of hearts with the calcium antagonists verapamil and diltiazem before the onset of total global ischaemia of 15 and 60 min duration inhibited myocardial noradrenaline loss following reperfusion (Nayler and Sturrock, 1984). Myocardial noradrenaline loss induced by 15 min global ischaemia and reperfusion was not inhibited when verapamil and diltiazem were added only on reperfusion or when the calcium content of the reperfusion buffer was reduced (Nayler and Sturrock, 1985).

However, perfusion of the isolated rat heart with calcium-free perfusate (with or without EGTA) prior to 20 min of global ischaemia did not reduce the noradrenaline overflow upon reperfusion suggesting that even ischaemic release may not be mediated by calcium (Schomig, Dart, Dietz, Mayer and Kubler, 1984). Neuronal uptake blockers such as desipramine, when added to the perfusate prior to ischaemia, reduced noradrenaline overflow during reperfusion after ischaemic periods of between 10 and 40 min. This led Schomig and co-workers to suggest that noradrenaline released from the sympathetic nerve

terminals by ischaemic periods of between 10 and 40 min was not due to exocytosis but the result of a calcium-independent active efflux of noradrenaline, using the same carrier mechanism normally responsible for neuronal uptake (Schomig et al, 1984; Schomig, Dart, Dietz, Kubler and Mayer, 1985).

The first study demonstrating an increased release of myocardial noradrenaline by the isolated rat heart during the acute ischaemic phase has recently been published by Carlsson, Abrahamsson and Almgren (1985). Using hearts labelled with tritiated noradrenaline, they were able to show an increased release of noradrenaline within 10-20 min of both regional (coronary occlusion) and global (90% flow reduction) ischaemia. However, using a similar model, other workers were unable to detect an enhanced output of noradrenaline following coronary occlusion, although washout of lactate from the ischaemic region was readily demonstrable (Daugherty, Frayn, Redfern and Woodward, 1986).

Increased release of noradrenaline from the ischaemic myocardium during reperfusion has also been demonstrated in the anaesthetized dog after 40 (Williams, Coker, Dean, Kane and Parratt, 1986) and 60 min (Lamontagne, Yamaguchi, Nadeau, De Champlain, Godin and Campeau, 1986) of coronary occlusion. Evidence regarding the release of noradrenaline into local venous blood draining the ischaemic myocardium during ischaemia is more contradictory. Early experiments demonstrated release of catecholamines as early as 2 min after coronary occlusion (Shahab, Wollenberger, Krause and Genz, 1972) but this finding was not supported by McGrath, Lim, Leversha and Shanahan (1981) who failed to detect elevated concentrations of noradrenaline

in the local coronary effluent after 10 min of occlusion. More recent studies have also indicated no spontaneous overflow of noradrenaline into the ischaemic venous effluent after 3 and 11 min of coronary occlusion (Forfar, Riemersma and Oliver, 1983; Forfar, Russell and Riemersma, 1985).

The general implication of the available evidence is that myocardial noradrenaline release does indeed occur during ischaemia, possibly by exocytosis although there is also some evidence of an active efflux in the rat. Despite the fact that noradrenaline release is only detectable during early coronary reperfusion in most studies, the kinetics of this release plus the metabolic and electrophysiological consequences of acute ischaemia support the view that at least some noradrenaline is released during ischaemia. Presynaptic inhibition of release and neuronal reuptake, processes which are enhanced by the drastically reduced coronary perfusion during ischaemia, may be responsible for the lack of increased overflow into the coronary venous effluent in the presence of an increased noradrenaline turnover during early ischaemia (Forfar et al, 1985).

1.7. Catecholamines and ischaemia-induced arrhythmias

The most pertinent question is perhaps not just whether catecholamines are released during ischaemia, but whether such a release initiates or sustains ventricular arrhythmias. Although plasma catecholamine levels in patients with acute myocardial infarction have been related to the severity of arrhythmias (Bertel et al, 1982), it is possible that the elevated plasma levels merely indicated a reflex response of the body's circulatory control system

to arrhythmias (Goldstein, 1981). In addition, clinical studies showing a beneficial effect with β-adrenoceptor blocking drugs (Multicentre International study, 1975; Norris, Brown, Clarke, Barnaby, Geary, Logan and Sharpe, 1984; The Norwegian Multicenter Study Group, 1981; Wilhelmsson, Wilhelmsen, Vedin, Tibblin and Werko, 1975) were all carried out in patients who survived the acute phase of myocardial infarction and bear little relevance to early arrhythmogenesis.

In experimental studies, a temporal association between increased plasma catecholamine levels and the development of early ventricular arrhythmias has been observed during coronary occlusion (Ceremuzynski, Staszewska-Barczak and Herbaczynska-Cedro, 1969; Kelliher, Widmar and Roberts, 1975). Gillis (1971) reported a consistent association between increased cardiac pre-ganglionic efferent sympathetic activity and severe ventricular arrhythmias after coronary occlusion, and it has been shown that normal neurosympathetic responsiveness is maintained in ischaemic tissue during the period corresponding to the early phase of arrhythmias (Forfar, Riemersma, Russell and Oliver, 1984).

The majority of experimental studies investigating the relationship between catecholamines and arrhythmias have employed surgical and pharmacological techniques to produce or inhibit adrenergic activation during ischaemia. The results and implications of some of these studies will be discussed in the following sections.

1.7.(1) Effects of sympathetic stimulation on arrhythmias

In anaesthetized dogs, ventricular fibrillation could be induced

by stimulation of central sympathetic centres in the posterior hypothalamus after coronary occlusion (Lown and Verrier, 1976). Stimulation of the stellate ganglia lowered the ventricular fibrillation threshold in the absence of ischaemia (Verrier, Thompson and Lown, 1974) and significantly increased in the incidence of ventricular fibrillation after coronary occlusion (Euler, Nattel, Spear, Moore and Scanlon, 1985).

Systemic administration of catecholamines has been shown to exacerbate arrhythmias during acute myocardial ischaemia (Harris, Otero and Bocage, 1971). Subepicardial infusion of noradrenaline, adrenaline and isoprenaline had a potent arrhythmogenic effect in the pig, both in the presence and absence of ischaemia (Podzuweit, 1982). In the isolated guinea pig heart, methoxamine has been shown to reverse the antiarrhythmic effects of catecholamine depletion during ischaemia (Penny, 1984).

In contrast to these studies, both noradrenaline and adrenaline have been found to protect against coronary occlusion induced arrhythmias in the anaesthetized rat when administered intravenously, either by continuous infusion (Parratt, Campbell and Fagbemi, 1981) or bolus injection (Marshall, Muir and Winslow, 1981). In the same model, however, isoprenaline increased the incidence of ventricular fibrillation (Marshall et al, 1981).

1.7.(2) Effects of surgical and chemical sympathectomy on arrhythmias

Chronic cardiac sympathetic denervation protected against coronary occlusion induced arrhythmias and mortality in the dog (Ebert, Vanderbeek, Allgood and Sabiston, 1970; Fowlis, Sang, Lundy,

Ahuja and Colhoun, 1974) but acute denervation was without effect (Ebert et al, 1970), suggesting that the beneficial effect was due to depletion of myocardial noradrenaline, which occurs after chronic denervation. It has been suggested recently, however, that chronic cardiac denervation may have an adverse effect on infarct size, an effect possibly related to an impaired collateral perfusion (Lavalle, Amano, Vatner, Manders, Randall and Thomas, 1985).

Supporting the view that myocardial noradrenaline depletion is beneficial, a study by Martin and Meesman (1985) showed that regional myocardial chemical sympathectomy in the dog with intracoronary injections of 6-hydroxydopamine had a significant antiarrhythmic effect during coronary occlusion. An antiarrhythmic effect with chemical sympathectomy has also been observed in the cat (Sheridan, Penkoske, Sobel and Corr, 1980), guinea-pig (Culling, Penny, Lewis, Middleton and Sheridan, 1984) and rat (Daugherty et al, 1986) heart. Although it has been suggested that the beneficial effect of pretreatment with 6-hydroxydopamine may be related to an increased myocardial content of glycogen (Daugherty et al, 1986), Culling et al (1984) showed that fasting the animals prior to study abolished this effect without altering the antiarrhythmic effect of chemical sympathectomy. More recently, it has been shown that pretreatment with the amino acid α-methyl-metatyrosine reduced myocardial noradrenaline content in the rat by over 90%, with no effect on glycogen and adenine nucleotide levels, and significantly reduced the incidence of ventricular fibrillation and mortality (Abrahamsson, Almgren, Carlsson and Svensson, 1985).

However Botting, Johnston, Macleod and Walker (1983) found that

combined adrenomedullectomy and 6-hydroxydopamine pretreatment had no marked effect on arrhythmias but inceased mortality after coronary occlusion in the conscious rat. Pretreatment with reserpine has also been found to increase the severity of arrhythmias and the incidence of mortality in the anaesthetized rat (Parratt et al, 1981). Neither of these studies, however, reported the effects of their treatments on the plasma or myocardial levels of catecholamines.

1.7.(3) Effects of β-adrenoceptor antagonists on arrhythmias

The experimental evidence regarding the effects of β-adrenoceptor antagonism on ischaemia induced arrhythmias is equivocal. Several agents, such as propranolol (Benfey, Elfellah, Ogilvie and Varma, 1984), timolol (Coker and Parratt, 1984), oxprenolol (Campbell, Parratt, Kane and Bullock, 1984) and sotalol (Patterson, Lynch and Lucchesi, 1984), have been shown to protect against arrhythmias following coronary occlusion in a variety of species. Labetalol was highly protective against ischaemia-induced arrhythmias in the cat (Pogwizd, Sharma and Corr, 1982) but had no significant effect in the rat (Botting et al, 1983) at similar doses.

Arguing against a significant role for β-adrenoceptors in arrhythmogenesis, propranolol (Botting et al, 1983), atenolol (Daugherty et al, 1986) and metoprolol (Lepran, Parratt, Szekeres and Wainwright, 1985) were ineffective in the rat. Daugherty et al (1986) found both optical isomers of propranolol to be eqipotent in suppressing arrhythmias, confuting the involvement of β-adrenoceptors and suggesting a local anaesthetic (Class 1) effect. The observed antiarrhythmic effect of sotalol has been attributed to its class III antiarrhythmic property (Patterson et al, 1984; Cobbe and Manley,

1985) and labetalol has been found to have both class I and class III antiarrhythmic actions (Dukes and Vaughan Williams, 1984). Curtis, Macleod and Walker (1985) have suggested that β-adrenoceptor antagonists may owe their protective effect to their ability to raise plasma potassium rather than myocardial receptor blockade. It has also been postulated that β-adrenoceptors mediate the chronotropic and inotropic but not the arrhythmogenic effects of catecholamines (Korczyn and Teplitsky, 1984).

1.7.(4) Effects of α-adrenoceptor antagonists on arrhythmias

The role of myocardial α-adrenoceptors in the development of ischaemia-induced arrhythmias is also not resolved. Sheridan, Penkoske, Sobel and Corr (1980) showed phentolamine and prazosin to have significant antiarrhythmic effects in the cat and suggested that an enhanced α-adrenergic responsiveness may occur during myocardial ischaemia. These workers later proposed an increase in the number of α-adrenoceptors in the ischaemic myocardium (Corr, Shayman, Kramer and Kipnis, 1981). The antiarrhythmic effect of α-adrenoceptor antagonism was also observed in the dog (Benfey et al, 1984) and the rat (Parratt et al, 1981) using prazosin and in the guinea pig with phentolamine and indoramin (Penny, Culling, Lewis and Sheridan, 1985).

In contrast, the strictly controlled study by Bolli, Fisher, Taylor, Young and Miller (1984) showed no beneficial effect on ischaemia-induced arrhythmias in the dog with phentolamine and prazosin and postulated that α-adrenergic mechanisms were unimportant in arrhythmogenesis. Daugherty et al (1986) found phentolamine and prazosin to be protective against arrhythmias in the isolated rat

heart but related this effect to their local anaesthetic properties. Indeed, two other α-adrenoceptor antagonists phenoxybenzamine and trimazosin, which had no discernible local anaesthetic properties, had no significant effect on the arrhythmias (Daugherty et al, 1986). Other electrophysiological studies on isolated heart tissue have also confirmed the local anaesthetic properties of phentolamine (Northover, 1983) and prazosin (Dukes and Vaughan Williams, 1984).

1.8. Possible arrhythmogenic mechanisms of catecholamines

The evidence presented suggests that local catecholamine release within the ischaemic myocardium may contribute to arrhythmogenesis, although the role of a general sympathetic activation is less clear and may prove protective in some models. The mechanism of the arrhythmogenic action of locally released noradrenaline, however, is far from clear, as indicated by the controversy regarding the effects of α- and β-adrenoceptor antagonists.

Since the discovery of a late increase in cyclic AMP (cAMP) in the ischaemic myocardium of the baboon, coinciding with the development of ventricular fibrillation, by Podzuweit, Dalby, Cherry and Opie (1978) the importance of cAMP as a "second messenger" in arrhythmogenesis has been extensively studied. Elevated cAMP levels in the ischaemic myocardium following coronary occlusion has also been demonstrated in cat (Corr, Witkowski and Sobel, 1978), dog (Ogawa, Ban, Kanayama and Ukai, 1983), pig and rat (Podzuweit, 1982) hearts. The arrhythmogenic effect of cAMP was probably mediated by enhanced calcium-dependent slow responses in the ischaemic myocardium where the fast sodium channel was blocked by elevated extracellular

potassium - an effect predisposing to re-entry arrhythmias (Opie, 1981).

More recent studies, however, shed doubt over the role of cAMP as a prime arrhythmogen. It has been shown in the anaesthetized rat that the occurence of coronary occlusion induced arrhythmias was not accompanied by a rise in cAMP in the ischaemic myocardium, although agents which elevated myocardial cAMP levels exacerbated the arrhythmias (Kane, Morcillo-Sanchez, Parratt, Rodger and Shahid, 1985). These workers, however, did observe a transient early rise in cAMP and suggested that rather than having a direct arrhythmogenic effect, cAMP might initiate a chain of further intracellular events which could subsequently lead to the genesis of arrhythmias. It has indeed been suggested that cAMP could stimulate tissue lipases and lead to the accumulation of potentially harmful lipid metabolites such as free fatty acids, acyl CoA, acylcarnitine and lysophospholipids (Opie, 1981). The possible involvement of these factors in arrhythmogenesis will be discussed later in this chapter.

A transient early increase in tissue cAMP content during ischaemia, which returned to the pre-ischaemic value prior to the development of arrhythmias, has also been observed using the isolated rat heart (Manning, Kinoshita, Buschmans, Coltart and Hearse, 1985). In this study, adrenaline elevated tissue cAMP and exacerbated arrhythmias induced by coronary occlusion but forskolin, which activates adenylcyclase independent of the β-adrenoceptor, had an antiarrhythmic effect despite producing an even greater increase in cAMP. The authors suggested that either the arrhythmogenic effect of catecholamines was not β-adrenoceptor mediated or some other consequence of β-adrenoceptor stimulation, that is not mediated

through adenylcyclase, was responsible for the arrhythmias. In the anaesthetized pig, metoprolol, propranolol and sotalol lowered tissue levels of cAMP prior to coronary occlusion but did not prevent the temporary increase in ischaemic tissue levels following coronary occlusion, and only metoprolol had a significant protective effect despite producing the highest cAMP levels following occlusion (Muller, Opie, Hamm, Peisach, Gihwala, Steyn and Basset, 1986).

An alternative mechanism by which catecholamines may contribute to arrhythmogenesis is the formation of potentially toxic products such as adrenochrome and free radicals by auto-oxidation (Singal, Beamish and Dhalla, 1983). Catecholamines have also been shown to potentiate thromboxane A_2 release from platelets in the isolated rat heart (Purchase, Dusting, Li and Read, 1986), an agent which has been implicated as an arrhythmogenic factor (Parratt and Coker, 1985).

1.9. Effects of α_2-adrenoceptor antagonism and neuronal uptake blockade on blood pressure and heart rate

In experimental animals, intravenous administration of yohimbine and idazoxan, the two α_2-adrenoceptor antagonists used in this study, produced similar biphasic effects on blood pressure and heart rate. Low doses of these compounds (0.01-0.3 mg/kg approximately) produced significant increases in blood pressure and heart rate whereas at doses of 1.0 mg/kg and above both drugs caused reductions in these parameters (Ramage and Tomlinson, 1985; Paciorek and Shepperson, 1985). Forfar et al (1983) also observed a significant reduction in mean arterial pressure with 1.0 mg/kg i.v. yohimbine. It has been suggested that an increased vagal tone may be responsible for the

reduction in heart rate with high doses of these drugs (Ramage and Tomlinson, 1985).

The neuronal uptake blocking agent desipramine has been shown to cause a transient increase in blood pressure without affecting heart rate when given intravenously at a 1.0 mg/kg dose in the rat (Graham, Stephenson and Pettinger, 1980). With the same dose, small but significant increases were observed in both blood pressure and heart rate in the dog (Yamaguchi et al, 1977).

1.10. Effects of α_2-adrenoceptor antagonism and neuronal uptake blockade on catecholamine release and ischaemia-induced arrhythmias

Intravenous administration of yohimbine at 1.0 mg/kg (Graham et al, 1980) and idazoxan at 0.3 mg/kg (Brown and Harland, 1984) have been shown to produce significant increases in arterial noradrenaline concentration in the rat, whereas desipramine at 1.0 mg/kg had no effect (Graham et al, 1980). Desipramine (1.0 mg/kg i.v.) also failed to produce a significant increase in the dog (Yamaguchi et al, 1977). The failure by desipramine to elevate plasma levels of noradrenaline, despite blocking neuronal uptake, is probably due to an inhibition of further release as a result of elevated noradrenaline levels in the synaptic cleft (Cousineau, Goresky and Rose, 1986).

The combination of α_2-adrenoceptor antagonism and neuronal uptake blockade caused large increases in arterial levels of noradrenaline in both the rat (Graham et al, 1980) and the dog (Forfar et al, 1985). The changes in plasma levels of catecholamines produced by these agents in the rat are probably mainly mediated by effects on neuronal release and removal as the adrenal medulla in

this species does not possess an uptake mechanism (Wakade and Wakade, 1984) or inhibitory α_2-adrenoceptors (Sharma, Wakade, Malhotra and Wakade, 1986).

Yohimbine and desipramine increased neurally evoked overflow of noradrenaline from the rat heart both during normal flow (Dart, Dietz, Kubler, Schomig and Strasser, 1983; Dart, Dietz, Hieronymus, Kubler, Mayer, Schomig and Strasser, 1984) and global ischaemia (Dart, Schomig, Dietz, Mayer and Kubler, 1984), despite a suppression of neurally mediated noradrenaline release during the ischaemic period. Similarly, both drugs enhanced noradrenaline overflow into the local coronary venous effluent during stimulation of the left stellate ganglion in the dog (Forfar et al, 1983; 1985).

Spontaneous noradrenaline release from the heart was not observed with either drug alone following coronary occlusion but their combination produced a significant spontaneous release from the ischaemic area (Forfar et al, 1985). The studies of Schomig et al (1984; 1985) using the isolated rat heart, however, showed that yohimbine did not alter the noradrenaline overflow after 20 min of global ischaemia but desipramine and a number of other neuronal uptake blockers significantly reduced it. As already mentioned, these workers have suggested that noradrenaline release from sympathetic nerve terminals during 10-40 min of ischaemia was not due to exocytosis, but the result of a carrier-mediated efflux using the same carrier that is normally responsible for transporting noradrenaline from the synaptic cleft back into the neuron (Schomig et al, 1984; 1985).

Forfar et al (1983) found that yohimbine increased the severity

and heterogeneity of conduction abnormalities in the ischaemic zone and increased the incidence of ventricular fibrillation following coronary occlusion from 22% to 56% in the dog. They later noted a similar increase in spontaneous ventricular fibrillation with desipramine (Forfar et al, 1985) but the effect of the combination of the two agents on arrhythmias was not reported. In the isolated rat heart, however, desipramine had a marked protective effect against coronary occlusion-induced arrhythmias and was a more potent antiarrhythmic than a variety of other agents such as propranolol, phentolamine, prazosin, lignocaine and quinidine (Daugherty et al, 1986). Using the same model, yohimbine has also been shown to have a significant antiarrhythmic effect, during both ischaemia and reperfusion (Thandroyen, Worthington, Higginson and Opie, 1983).

There appears to be considerable disparity regarding the effects of α_2-antagonism and neuronal uptake blockade on ischaemia-induced noradrenaline release and arrhythmias in the in vivo and in vitro models described above. Further studies are clearly required in order to delineate the importance of presynaptic inhibition and neuronal uptake processes during acute myocardial ischaemia, and the effects of their blockade on the consequences of ischaemia. The present study was initiated in an effort to clarify these points.

1.11. Plasma potassium concentration and ischaemia-induced arrhythmias

Nordrehaug and von der Lippe (1983) have shown a striking inverse relationship between plasma potassium and the incidence of ventricular fibrillation in patients with acute myocardial infarction, observing a five times higher incidence of ventricular

fibrillation in those patients who, on admission, had a low plasma potassium concentration. They proposed that plasma potassium was an inverse predictor of the occurence of ventricular arrhythmias in acute myocardial infarction (Nordrehaug, Johannessen and von der Lippe, 1985).

This proposal has been supported by experimental findings in the rat. Curtis, Johnston and Walker (1985) investigated the effects of raising and lowering plasma potasium levels on coronary occlusion-induced arrhythmias and discovered an inverse correlation between the incidence and severity of arrhythmias and plasma potassium concentration. These workers have also suggested that the antiarrhythmic effects of various ablations in the central nervous system of rats could be mediated by elevated plasma potassium levels (Curtis, Macleod and Walker, 1985). Using the isolated rat heart, Daugherty, Mohamed and Woodward (1983) showed that by increasing the potassium concentration in the perfusate they could reduce the severity and incidence of coronary occlusion-induced arrhythmias. In support of these studies, reducing the plasma potassium concentration in anaesthetized rats by pretreatment with furosemide increased the incidence of ventricular fibrillation following coronary occlusion from 13% to 86% (Abrahamsson and Almgren, 1981).

In conclusion, there appears to be unequivocal clinical and experimental evidence suggesting that hypokalaemia can exacerbate ischaemia-induced arrhythmias and mild hyperkalaemia may be protective against these arrhythmias.

1.12. Effects of catecholamines on plasma potassium concentration

Although the maintenance of chronic potassium balance is primarily regulated by the kidney, various extrarenal mechanisms are also operative. One of the most important factors involved in such extrarenal regulation is circulating catecholamines (for review see Bia and De Fronzo, 1981).

The major effect of catecholamines on plasma potassium is mediated by β_2-adrenoceptors in skeletal muscle, the stimulation of which results in an increased cellular uptake of potassium and a reduced plasma concentration (Lockwood and Lum, 1974; Brown, Brown and Murphy, 1983). However, infusion of adrenaline has a biphasic effect in various species, with an initial rise in plasma potassium followed by a sustained decrease (Bia and De Fronzo, 1981). The initial rise in plasma potassium probably results from an α-adrenoceptor mediated potassium release by the liver and can be mimicked by α-adrenoceptor agonists and blocked by α-adrenoceptor antagonists (Bia and De Fronzo, 1981). In addition, the stimulation of α-adrenoceptors with phenylephrine has been shown to impair the extrarenal disposal of an acute potassium load in healthy volunteers (Williams, Rosa, Silva, Brown and Epstein, 1984). It has also been suggested, however, that adrenaline-induced potassium release and the resulting transient hyperkalaemia may be mediated through both α- and β-adrenoceptor stimulation (Todd and Vick, 1971; Coats, 1985).

Supporting a β-adrenoceptor mediated hypokalaemia and a weaker α-adrenoceptor mediated hyperkalaemia by circulating catecholamines, an inverse correlation has been observed between plasma adrenaline and potassium concentrations in patients with acute myocardial infarction prior to treatment. Following treatment with the non-

selective β-adrenoceptor antagonist timolol, however, the plasma levels of both adrenaline and noradrenaline were positively related to increases in plasma potassium (Nordrehaug, Johannessen, von der Lippe and Myking, 1985).

It is clear that pharmacological interventions that interfere with adrenoceptors and those that alter circulating catecholamine levels may have indirect effects on ischaemia-induced arrhythmias via their effects on the plasma concentration of potassium. It has indeed been suggested that β-adrenoceptor antagonists may reduce arrhythmias by raising plasma potassium rather than by blocking myocardial β-adrenoceptors, as mentioned earlier in this chapter (Curtis, Macleod and Walker, 1985).

1.13. Other biochemical factors implicated in arrhythmogenesis

In addition to catecholamines, several other biochemical factors have also been implicated in the development of ischaemia-induced arrhythmias. These will be discussed briefly in the following sections.

1.13.(1) Prostanoids

There is evidence that the balance between the local generation and release of thromboxane and prostacyclin may be important in arrhythmogenesis during myocardial ischaemia (Coker, 1982; Coker and Parratt, 1985). Early release of TxB_2 and 6-keto $PGF_{1\alpha}$, the major metabolites of thromboxane and prostacyclin respectively, into local coronary venous blood from the ischaemic area has been demonstrated in the dog following coronary occlusion (Coker, Parratt, Ledingham

and Zeitlin, 1981). The incidence of ventricular ectopic activity was positively correlated with TxB_2 release but negatively correlated with 6-keto $PGF_{1\alpha}$ release. Indeed, the inhibition of thromboxane synthesis, blockade of thromboxane receptors, local coronary infusion of prostacyclin or its stable analogue iloprost, and promotion of local myocardial prostacyclin generation with nafazatrom have all been shown to protect against arrhythmias (for review see Parratt and Coker, 1985). A disturbed ratio of 6-keto $PGF_{1\alpha}$ to TxB_2 in favour of TxB_2 has also been found in plasma from patients with acute myocardial infarction (Friedrich, Lichey, Nigam, Priesnitz and Wegscheider, 1985). Although the effects of thromboxane and prostacyclin are probably mainly mediated by their effects on the vasculature and platelets, prostacyclin may provide added protection by inhibiting noradrenaline release during ischaemia (Schror and Funke, 1985; Lanier and Malik, 1985).

1.13.(2) Free fatty acids

A possible association between circulating free fatty acids (FFA) and arrhythmogenesis was first noted in patients with acute myocardial infarction by Oliver and his colleagues (Oliver, Kurien and Greenwood, 1968). They later showed that ischaemia-induced arrhythmias could be exacerbated by elevating FFA levels using intralipid/heparin in dogs (Kurien, Yates and Oliver, 1971). Long chain saturated fatty acids have also been shown to potentiate the effect of hypoxia in lowering the ventricular fibrillation threshold in the rabbit heart (Murnaghan, 1981).

During ischaemia β-oxidation of FFA is inhibited resulting in increased tissue levels of long chain acyl CoA in the presence of

increased FFA availability. This could lead to the inhibition of adenine nucleotide translocases and a reduced transport of ATP from mitochondria to cytosol (Shug, Shrago, Bittar, Folts and Koke, 1975), leading to limited ATP availability for electrophysiological processes. Elevated FFA may also cause wastage of the limited available oxygen by increased triglyceride synthesis (Kurien and Oliver, 1970) and these processes could predispose the ischaemic heart to arrhythmias. Accumulation of amphiphilic long chain acylcarnitines has also been implicated in FFA-induced arrhythmogenesis (Corr and Sobel, 1983).

Although promoting glycolysis over FFA oxidation has been suggested to protect against arrhythmias (Russell, 1982) and ischaemic damage (De Leiris, Opie and Lubbe, 1975), products of anaerobic glycolysis such as lactate may promote arrhythmias by shortening action potential duration (Saman and Opie, 1984) and accelerate ischaemic damage to the myocardium (Neely and Grotyohann, 1984).

1.13.(3) Lysophospholipids

Phospholipid metabolites have been implicated in ischaemia induced arrhythmogenesis since the accumulation of lysophospholipids in the ischaemic myocardium was first demonstrated (Sobel, Corr, Robison, Goldstein, Witkowski and Klein, 1978). Although it was initially suggested that an increased phospholipase A_2 activity may be responsible for this accumulation during ischaemia, this was not supported by experimental results and current belief is that a depressed lysophospholipid metabolism is of greater importance (Corr

and Sobel, 1983).

Lysophospholipids have been shown to produce electrophysiological derangements in isolated canine Purkinje fibres analogous to those seen in ischaemic tissue in vivo (Corr, Cain, Witkowski, Price and Sobel, 1979). Indeed, arrhythmias leading to ventricular fibrillation have been induced by intracoronary infusion of lysophophatidylcholine in the cat (Bentham, 1986). Lysophospholipids are thought to produce their arrhythmogenic effect by incorporating into the sarcolemma and compromising normal membrane function (Corr and Sobel, 1983).

1.13.(4) Free radicals

There is current interest in the possible role of oxygen free radicals such as the superoxide anion in myocardial injury and arrhythmogenesis during ischaemia and reperfusion. Free radicals, by virtue of an unpaired electron, can react with virtually all cell components with deleterious consequences on cellular function and integrity.

Because of the low availability of oxygen, free radicals probably do not play a major role in ischaemia-induced arrhythmias but may be important in the genesis of reperfusion arrhythmias. In support of this theory, Woodward and Zakaria (1985) found that free radical scavengers and antioxidants protected the coronary artery ligated isolated rat heart against reperfusion-induced arrhythmias whereas at the same concentrations these agents were unable to protect against ischaemia induced arrhythmias (Mohamed, 1985). An increased free radical production during ischaemia, however, has been demonstrated in the dog after coronary occlusion (Rao, Cohen and

Mueller, 1983).

Although oxygen free radical generation may not be of major importance during ischaemia, changes occurring during this period such as the conversion of xanthine dehydrogenase to xanthine oxidase, accumulation of the ATP metabolite hypoxanthine, activation of the complement system and generation of chemotactic factors, and increased arachidonic acid metabolism may all facilitate the formation of free radicals upon restoration of physiological concentrations of oxygen (Werns, Shea and Lucchesi, 1986).

1.13.(5) Others

In addition to the factors already discussed, several other endogenous chemicals have been implicated in arrhythmogenesis. The histamine H_2-receptor antagonist SKF 93479 has been found to protect against arrhythmias induced by coronary artery occlusion in dogs and rats (Dai, 1984) and the possible involvement of histamine in arrhythmogenesis has been discussed in a recent review by Wolf and Levi (1986). Protection against ischaemia-induced arrhythmias has also been observed with antagonists at 5-HT (Coker, Dean, Kane and Parratt, 1986) and opioid (Fagbemi, Lepran, Parratt and Szekeres, 1982; Huang, Lee, Wong, Zhan and Zhao, 1986) receptors, suggesting possible arrhythmogenic roles for endogenous 5-HT and opioid peptides. It is not clear, however, whether the protective effects of such antagonists were due to blockade of myocardial receptors or the result of a non-specific local anaestheic effect (Coker and Parratt, 1985).

1.14. The rat as an experimental model of myocardial ischaemia

For comparison with man, no species is ideal for studying the effects of drugs on the ischaemia/infarction sequence, for the human heart changes its vasculature during ageing. While atheroma-free human hearts have few collaterals, old atheromatous hearts may rely heavily on collaterals. The rat heart has low collateral flow and consequently a rapid and complete infarct can be produced by coronary artery occlusion, a situation analogous to healthy young man (Schaper, 1984). Ischaemia-induced arrhythmias in the rat follow a similar time course to those observed in man and other frequently used animal species, with early and late phases of ectopic activity (Botting et al, 1986). Indeed, pharmacological agents frequently used in man to control cardiac arrhythmias are also effective in the coronary artery ligated rat (Kane, McDonald and Parratt, 1981). In addition, the price and relative availability of the rat allows for much more extensive investigation in terms of the number of animals used and the number of agents and doses tested.

The two major criticisms directed against the use of rats in investigating the electrical consequences of myocardial ischaemia have been the lack of an isoelectric ST segment in the rat electrocardiogram and the spontaneous reversion of venticular fibrillation observed in this species. The former is the result of the absence of a prolonged plateau phase in the rat action potential due to the fast kinetics of the slow inward current (I_{si}) and the latter possibly related to the small size of the heart, resulting in a smaller number of wavefronts in ventricular fibrillation with a subsequently increased probability of spontaneous defibrillation (Botting et al, 1986). Despite these drawbacks, however, the coronary artery ligated

rat has proved to be a useful and reliable model of ischaemia-induced arrhythmias and it has been argued that such criticism should not negate its continued use (Botting et al, 1986).

1.15. Aims of the study

The main objectives of this study were to investigate the relationship between sympathetic activity and ischaemia-induced arrhythmias, and to clarify the importance of presynaptic inhibition and neuronal uptake in determining the consequences of ischaemia, using coronary artery occlusion in the anaesthetized rat. To this end, the effects of neuronal uptake blockade and α_2-adrenoceptor antagonism (both alone and in combination) on haemodynamic parameters and arrhythmias were studied. The effects of these drugs on plasma levels of noradrenaline and adrenaline following coronary occlusion were monitored as an indicator of general sympathetic activity. Finally, any relationship between the effects of these agents on plasma potassium levels and their effects on plasma catecholamine levels and arrhythmias was investigated using potassium-selective electrodes.

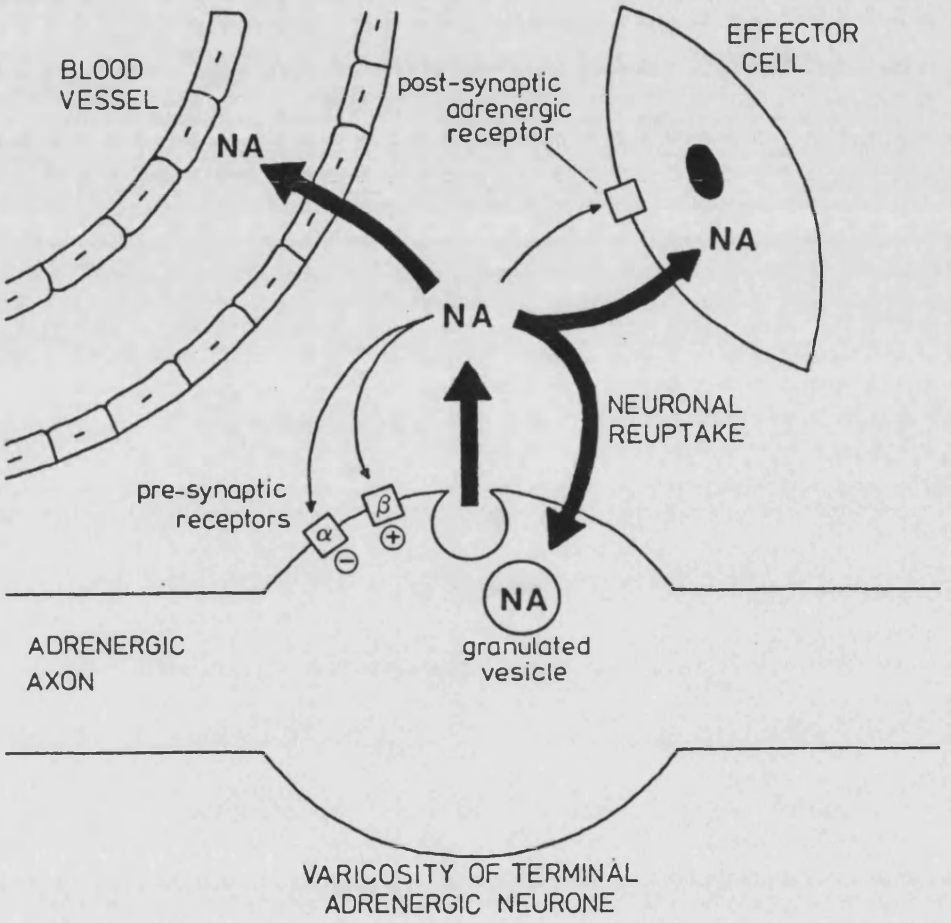


Figure 1 Diagrammatical representation of noradrenaline release and removal mechanisms at sympathetic nerve terminals.

Chapter 2

MATERIALS AND METHODS

2.1. The anaesthetized rat model for coronary artery occlusion-induced arrhythmias

The model described by Clark et al (1980) was used throughout this study to investigate the effects of drugs on arrhythmias induced by coronary artery occlusion.

2.1.(1) Experimental procedure

Male Wistar rats (University of Bath strain), weighing 250-350 g, were anaesthetized with sodium pentobarbitone (Sagatal, May and Baker), 60 mg/kg intraperitoneally. The trachea was cannulated to allow artificial ventilation. Systemic arterial blood pressure was recorded from the left common carotid artery using a physiological pressure transducer (Gould P 23 ID) coupled to a pressure processor amplifier (Gould 13-4615-52), via a polythene cannula filled with heparinised saline (50 U/ml). A saline filled polythene cannula was placed in the left femoral vein for administration of drugs. The lead II electrocardiogram (ECG) was recorded using standard limb leads coupled to an ECG/biotach amplifier (Gould 13-4615-65). Arterial blood pressure, ECG and heart rate, which was derived from the ECG signal by the ECG/biotach amplifier, were continuously recorded at a slow chart speed (5 mm/min) on a 3-channel thermal writing recorder (Gould 3000S). Blood pressure and ECG were also displayed on a 2-channel ink writing recorder (Gould 2200S) whose chart speed could be adjusted rapidly to enable characterization and evaluation of the various arrhythmias. Body temperature was maintained at 37.5^{o}C using a homeothermic blanket and a rectal probe (CFP 8142).

The chest was opened by left thoracotomy at the fifth

intercostal space and the adjoining ribs were sectioned close to the left margin of the sternum. Artifical ventilation with room air was begun immediately before opening the chest at a stroke volume of 2 ml/100 g body weight and a rate of 54 strokes/minute. After opening the pericardium, the heart was exteriorized by exerting pressure on the lowest part of the rib cage. The heart was held between the thumb and index finger of the left hand while a 4/0 braided silk suture (Mersilk W191, Ethicon) attached to a 16mm curved needle (Arnold Veterinary Products) was placed under the left coronary artery as described by Selye et al (1960). During this procedure, the heart did not remain exteriorized for more than a few seconds and was rapidly replaced in the thoracic cavity. The ends of the suture were threaded through a 2 cm length of narrow polythene tubing to form a snare which could be tightened when required. The animal was then allowed to recover for 10 minutes.

If there were no arrhythmias and the mean arterial pressure remained above 70 mmHg in that period, the animal was given the drug under investigation or an equivalent volume of physiological saline in control and sham-occlusion groups, via the femoral vein cannula. Drug solutions were prepared in such concentrations that the volume injected did not exceed 0.18 ml "washed in" with 0.2 ml saline. Five minutes after drug or saline administration, the ligature was tightened by gently pushing the polythene tubing onto the left ventricular wall while pulling the ends of the suture. The tightened snare was secured in place with a bulldog clip. In sham-occluded animals the ligature was not tightened but the suture was lightly pulled and released. In experiments investigating the effects of

drugs when given following coronary occlusion, the drug (or saline in controls) was injected into the femoral vein 2 minutes after tightening of the ligature.

Blood pressure, heart rate and ECG recordings were continued for 20 minutes after coronary occlusion or sham occlusion when assessing the incidence and severity of post-occlusion arrhythmias. Changes in blood pressure, heart rate and the amplitude of the ECG signal were noted from the slow speed recording. The type and severity of arrhythmias and other electrocardiographic changes were evaluated from the high speed blood pressure/ECG recordings.

At the end of each experiment the rat was intravenously injected with a dye solution (patent blue violet, Sigma) to confirm successful occlusion of tne left coronary artery, before administering a lethal overdose of anaesthetic. Any animals which did not exhibit a clear contrast between the "ischaemic" left ventricle and the rest of the heart, which would be coloured dark blue, were excluded from the study. In over 95% of the experiments in this study the coronary artery was successfully occluded.

2.1.(2) Evaluation of haemodynamic changes and arrhythmias

Blood pressure and heart rate values were noted from the recordings at pre-determined time intervals. These were: just before drug administration, at one minute intervals following administration until the time of coronary occlusion, and 5 minutes after coronary occlusion. All blood pressure values were calculated as the mean arterial pressure (MAP) from the following formula:

$$MAP = DP + \frac{PP}{3}$$

where DP = diastolic pressure

PP = pulse pressure (systolic pressure-diastolic pressure).

The extent of arrhythmic activity was assessed by counting the total number of premature ventricular contractions (PVC), including those occuring as ventricular tachycardia (VT), and noting the incidence of VT, ventricular fibrillation (VF) and mortality in the experimental groups. The onset of the first episode and the total duration of all episodes were also recorded for VT and VF. If at any time during the period of coronary occlusion blood pressure dropped below 20 mmHg and remained there for over 3 minutes, as a result of sustained VT or VF, the animal was considered to be dead and the experiment terminated.

VT was defined as any run of seven or more consecutive premature ventricular contractions accompanied by a precipitous fall in blood pressure and VF as an irregular ECG pattern with the blood pressure approaching zero. Examples of the various types of arrhythmias observed in this model are shown in Chapter 4 (figure 19).

When investigating the relationship between haemodynamic parameters (mean arterial pressure and heart rate) at the time of coronary occlusion and the severity of subsequent arrhythmias it was desirable to have an overall index of arrhythmic activity. Therefore, an arrhythmia score was devised on an arbitrary scale as follows: 1 point per 100 PVCs, 1 point per 20s VT, 1 point per 10 s VF and 2 points if the animal died.

2.1.(3) Collection of blood samples

Arterial blood samples for catecholamine analysis were collected from the carotid cannula 3 minutes after coronary occlusion or sham-occlusion. This time point (which precedes the development of arrhythmias) was chosen to investigate any causal relationship between the plasma catecholamine concentration and development of arrhythmias. About 4 ml of blood was collected into chilled glass tubes containing 100 µl of 0.2M reduced glutathione (GSH, Sigma) and 100 µl of heparin (1000 U/ml). The blood was centrifuged at 2000 rpm for 10 minutes at 4°C (MSE Chillspin) to separate the plasma phase and the plasma samples were stored frozen at -25°C until assayed. The experiments were terminated after the collection of blood samples with a lethal overdose of anaesthetic.

In some groups, blood samples were collected by the same route at the end of the 20 minute occlusion period for the measurement of plasma potassium levels. Approximately 1ml of blood was collected and the plasma separated by centrifugation at 2000 rpm for 10 minutes (IEC Centra-7) for analysis by flame photometry.

2.2. Determination of plasma catecholamines

Plasma samples were assayed for noradrenaline and adrenaline by a modification of the method described by Eriksson and Persson (1982), utilizing alumina extraction and analysis of the extracts by high performance liquid chromatography (HPLC).

2.2.(1) Preparation of alumina (aluminium oxide)

Aluminium oxide was activated in order to obtain the grade recommended by Anton and Sayre (1962). 100 grams of aluminium oxide

(neutral, Brockmann grade 1, BDH) was added to 500 ml of 2M HCl in a beaker, covered and heated to 90-100°C on a hot plate. The temperature was monitored with a thermometer and the aluminium oxide continuously stirred with a magnetic stirrer for 45 minutes at this temperature. The aluminium oxide was then allowed to settle and the supernatant fluid, yellow in colour, was discarded along with the finer particles of alumina.

The precipitate was washed twice with fresh 250 ml portions of 2M HCl at 70°C for 10 minutes, discarding the supernatant each time. In the final acid wash, the alumina was stirred in 500 ml of 2M HCl at 50°C for 10 minutes. After decanting the HCl, the precipitate was washed repeatedly (20-25 times) with fresh 200 ml volumes of distilled water until the supernatant had a pH of 3.4.

Finally, the aluminium oxide was transferred to an evaporating dish and heated at 120°C for 1 hour and at 200°C for a further 2 hours in an oven. The alumina was then stored in a capped glass bottle in a dessicator at room temperature.

2.2.(2) Extraction procedure

1.5 ml portions of plasma samples were placed in Eppendorf tubes to which 48 pmoles of the internal standard, 3,4-dihydroxybenzylamine (DHBA, Sigma) dissolved in 0.2 M perchloric acid, had been added. The samples were well mixed and transferred to plastic tubes containing 50 µl of 50 mM reduced glutathione (GSH, Sigma), 50 µl of 300 mM ethylenediaminetetra-acetic acid disodium salt (EDTA, BDH) and 25 mg of activated alumina. 0.2 ml of 1M Tris buffer (pH 8.6), prepared by mixing 1 M solutions of Trizma base and Trizma

hydrochloride (both from Sigma), was then added to the samples. The tubes were capped, manually shaken and mixed on a rotary mixer (Luckham) for 20 minutes.

Following mixing, the tubes were centrifuged at 1000 rpm for 5 minutes (IEC Centra-7) to settle the alumina and the supernatant was discarded. The alumina was washed 3 times with a 3 mM EDTA solution (pH 7.0), centrifuging between washes and discarding the supernatant.

After the final wash the alumina was transferred to the sample compartment of a BAS microfilter assembly with a regenerated cellulose membrane (Anachem) using a Pasteur pipette, with the supernatant (3 mM EDTA) as the transfer medium. Any alumina particles adhering to the pipette were washed out with fresh 3 mM EDTA. The microfilter assemblies were centrifuged at 1000 rpm for 5 minutes and the alumina dried by gradually increasing the speed to 1800 rpm and spinning at that speed for a further 5 minutes.

The eluate was then discarded and new receiving tubes attached to the miscrofilter assemblies. 120 µl of 0.2 M perchloric acid was added to the alumina in the sample compartment and the microfilters were left standing for 15 minutes. The eluates were collected by centrifuging the microfilter assemblies at 1800 rpm for 10 minutes and stored frozen at -25^{o}C until HPLC analysis.

The extraction procedure was later simplified in order to save time by eliminating the microfilter stage. The procedure was exactly the same as described above except after the final wash with 3 mM EDTA the samples were centrifuged and the supernatant was discarded. 200 µl of 0.2 M perchloric acid was then added to the alumina, mixed for 10 minutes and the tubes centrifuged at 1000 rpm for 5 minutes. 150 µl of the supernatant was then pipetted out and stored frozen at

-25°C until HPLC analysis.

2.2.(3) Assay of plasma extracts for catecholamines by high performance liquid chromatography (HPLC)

The noradrenaline (NA) and adrenaline (A) contents of the perchloric acid extracts were determined by reversed phase, ion-pair HPLC with electrochemical detection.

The liquid chromatograph was composed of an LDC Model III Constametric pump, a Rheodyne 7125 injection valve with a 100µl loop, a 25 cm x 4.6 mm i.d. stainless steel analytical column packed with 5 µm diameter Spherisorb-ODS particles (Anachem) and a BAS LC-4A amperometric detector. The detector was operated at + 0.65V with an Ag/AgCl reference electrode (BAS RE-1) and a glassy carbon working electrode. The flow rate of the mobile phase was maintained at 1.0 ml/min.

The mobile phase was an acetate/citrate buffer (pH 5.2) containing 5% methanol (HPLC grade) and 0.5 mM 1-octanesulphonic acid sodium salt (HPLC grade, Fisons), the ion pairing agent. The composition of the buffer was 100 mM sodium acetate, 60 mM sodium hydroxide and 40 mM citric acid. The water used for the mobile phase was deionized and filtered by a Millipore Milli-Q water purification system. Prior to use, the mobile phase was filtered under vacuum and degassed by bubbling helium through the solution for 15 minutes.

Chromatograms were recorded on a JJ Instruments flat-top recorder. Typical chromatograms from a plasma extract and a non-extracted standard solution are shown in figure 2. Peak heights were measured manually and peak height ratios (NA/DHBA and A/DHBA) calculated.

2.2.(4) Calibration

Aliquots of pooled plasma were spiked with known amounts of noradrenaline and adrenaline. After the addition of the internal standard DHBA, the samples were extracted and assayed by HPLC as previously described. Blank samples of pooled plasma were also analysed to enable correction for endogenous catecholamines present in the plasma.

The peak height ratios (NA/DHBA and A/DHBA) were plotted against plasma concentration to obtain calibration curves for both catecholamines. Typical calibration curves are shown in figure 3. These curves were used to convert peak height ratios to concentration values when analysing plasma samples.

2.2.(5) The linearity of the extraction procedure and detector response

The linearity of the extraction procedure was tested by spiking aliquots of pooled plasma with increasing amounts of noradrenaline and adrenaline and putting these samples through the procedure. The internal standard, DHBA, was not added to the samples at the beginning but was included in the 0.2 M perchloric acid used for the final elution, at the appropriate concentration. The extracts were then analysed by HPLC and the peak height ratios calculated. The results from the spiked samples were corrected for endogenous catecholamines using the results from blank pooled plasma samples.

The linearity of the detection system was evaluated by injecting standard solutions directly into the chromatographic system. The solutions contained increasing amounts of noradrenaline and adrenaline and a fixed concentration of DHBA in 0.2 M perchloric

acid.

Both the extraction procedure and the detector response were found to be linear within the range corresponding to plasma concentrations of 2.5-80 pmol/ml for noradrenaline and 5-160 pmol/ml for adrenaline ($r > 0.99$).

2.2.(6) The precision of the extraction procedure and chromatography

The precision of the extraction procedure and chromatography was evaluated by processing aliquots of plasma containing known amounts of noradrenaline and adrenaline, as in section 2.2(4). Inter- and intra-assay coefficients of variation (c.v.), calculated using spiked samples containing 5 pmol/ml noradrenaline and 10 pmol/ml adrenaline, were as follows (n=3 for all values).

	Inter-assay c.v.	Intra-assay c.v.
Noradrenaline	13.0%	11.2%
Adrenaline	14.2%	8.5%

2.2.(7) Calculation of recovery

The recovery of noradrenaline and adrenaline was calculated by comparing the peak height ratios obtained from the two procedures in section 2.2.(5), i.e. after alumina extraction of spiked samples and after direct injection of appropriate standards into the chromatograph. The recovery of DHBA was calculated by comparing the peak height ratios obtained during the construction of calibration curves (section 2.2.(4)) with those obtained when the DHBA was added during the final elution (section 2.2.(5)).

The recoveries for noradrenaline, adrenaline and DHBA were found

to be 61%, 61% and 64% respectively. The removal of the microfilter stage from the extraction procedure had no appreciable effect on these values.

2.3. Determination of plasma potassium by flame photometry

The concentration of potassium in plasma samples was determined using a Corning flame photometer (model 405) according to the manufacturer's instructions. Briefly, diluent concentrate (Corning 001 56 181K) was diluted 1:1000 with deionized water from a Millipore Milli-Q water purification system. The plasma samples and the 5 mM plasma potassium standard (Corning 001 56 015J) were then diluted 1:100 with the diluent (50 µl in 5 ml). Blank adjustment and calibration were carried out using the diluent and the standard potassium solution respectively. The diluted plasma samples were then aspirated and their concentrations noted from the meter readings. The calibration was checked after every 5 samples and duplicate readings were taken for all samples.

2.4. Continuous monitoring of venous potassium by ion-selective electrodes

A modified design of the electrode developed by Dr D. M. Band's group at the Sherrington School of Physiology, St. Thomas' Hospital Medical School, London (Treasure and Band, 1977; Linton, Lim and Band, 1982) was used in this study for continuous intravascular monitoring of potassium. Descriptions of the principle of the method and the electrode structures will first be given, followed by a detailed account of construction and experimental use.

2.4.(1) Principle of the method

The measurement of potassium with an ion-selective electrode depends on measuring the membrane potential established when a selectively permeable membrane is used to separate two dissimilar solutions, in this case venous blood and the internal filling solution. The membrane potential is proportional to the natural logarithm of the activity ratio of the potassium ion across the membrane and is predicted by the Nernst equation:

$$E = E^{o} + RT/F \ln a_o/a_i$$

where E = measured potential of the electrode

E^{o} = standard electrode potential (constant for each electrode)

R = gas constant

T = absolute temperature

F = Faraday's number

and a_o and a_i are the activities of the potassium ion outside and inside the membrane respectively.

Converting from the natural logarithm to the base 10 logarithm:

$$E = E^{o} + 2.3\ RT/F \log a_o/a_i$$

and entering the numerical values;

$$E = E^{o} + 61.6 \log a_o/a_i \quad \text{at } 37.5^{o}C$$

If the outer surface of the membrane is exposed to blood and the inner surface to the standard filling solution of unchanging potassium ion activity, then the potential across the membrane changes with the logarithm of the potassium activity in the blood.

Band, Kratochvil, Poole-Wilson and Treasure (1978) have established that the relationship between activity measured by these

electrodes and the actual concentration measured by flame photometry is linear. Therefore, the electrodes can be calibrated in concentration terms using potassium standards made up in a background of physiological saline (NaCl, 150 mM) in which the activity coefficient of potassium is constant and similar to that in the blood plasma (Band, Kratochvil and Treasure, 1977). The voltages recorded from the electrodes can then be interpreted in terms of plasma potassium concentration after logarithmic conversion.

2.4.(2) Electrode structures

The sensing electrode consisted of a 10 cm length of polyvinyl chloride (PVC) tubing (o.d. 1 mm, Esco (Rubber) Ltd) with a porous ceramic plug fitted into its distal end to support the ion-selective membrane. The lumen of the tubing was filled with the internal reference solution (100 mM KCl) and contained a chloridised silver wire acting as an internal reference electrode. The proximal end of the tubing was sealed and the internal reference electrode soldered onto a length of insulated copper wire which joined it to an electrical connector (figure 4,a).

The external reference electrode was a silver/silver chloride pellet electrode (Clark Electromedical) inserted into a modified Gillette 1 ml syringe filled with physiological saline. The proximal end was sealed and the electrode connected to an electrical connector by insulated copper wire. A polythene cannula was attached to the distal end via a 3-way tap, both filled with saline, forming a salt bridge linking the venous blood to the external reference electrode when inserted into the inferior vena cava. This arrangement had

the advantage of enabling intravenous administration of drugs via the 3-way tap when required (figure 4,b).

2.4.(3) Preparation of the potassium-selective membrane

The membrane mixture used consists of a combination of valinomycin and potassium tetraphenylborate, incorporated in a polyvinyl chloride (PVC) matrix by the use of the organic solvents nitrobenzene, dioctyladipate and tetrahydrofuran. The composition of the membrane, described by Band *et al* (1977) was as follows:

Component	Amount
Valinomycin (Sigma)	1.5 mg
Potassium tetraphenylborate	0.025 mg
Dioctyladipate (Merck)	150 mg
Nitrobenzene (BDH)	50 mg
PVC powder (BDH)	75 mg
Tetrahydrofuran (Fisons)	3 ml

Potassium tetraphenylborate (KTPB) was prepared by precipitation from sodium tetraphenylborate (tetraphenylboron sodium, Sigma). Briefly, an excess of saturated KCl was added to a 40 mg/ml solution of sodium tetraphenylborate. The resulting precipitate of KTPB was filtered out and washed, first with a KCl solution and then with distilled water. The KTPB was dried at 40^{o}C in an oven before storage in a capped glass vial at room temperature.

The membrane mixture was prepared in a clean glass pot with the components added in the following order: KTPB, valinomycin, PVC, nitrobenzene and dioctyladipate. The latter two, being liquids, were dispensed by fine tipped glass Pasteur pipettes. Finally, 3 ml of

tetrahydrofuran was added and the resultant mixture stirred magnetically with a nylon covered "flea" for 1 hour. After this, the mixture was left in a fume cupboard, loosely covered with a piece of filter paper, to evaporate slowly over 24 hours. This produced a clear solid residue consisting of PVC impregnated with valinomycin and KTPB which could be re-dissolved in a small amount of tetrahydrofuran to produce a thick viscous preparation suitable for dip-casting onto PVC tubing.

2.4.(4) Dip-casting of the membrane onto PVC tubing

Porous ceramic plugs (gift from Simonsen & Weel Ltd) were used to support the potassium-selective membrane at the tip of the PVC tubing. The plugs were inserted about 1 mm into the lumen of the PVC tubing, leaving a small portion protruding beyond the end. This was then rubbed with fine sandpaper to produce a smooth, rounded tip. A small amount of tetrahydrofuran was added to the membrane mixture prepared as described in section 2.4.(3) and magnetic stirring performed until a thick viscous preparation was obtained. The tip of the PVC tubing containing the ceramic plug was dipped several times into this mixture so as to coat the plug with a membrane which was fused to the PVC tubing. This membrane was allowed to dry for 24 hours.

2.4.(5) Preparation of the silver/silver chloride internal reference electrodes

Diamel-coated silver wire (0.005 in diameter, Johnson Matthey Metals Ltd) was used for the preparation of these electrodes. The varnish was stripped from the terminal 1.5 cm portions of 12 cm lengths of silver wire using a commercial paint stripper

(Polystrippa, Polycell Products). After washing in distilled water, cathodic cleaning was performed. This was achieved by dipping the wires in 0.1 M HCl and applying 3 volts to the stripped wires as the cathode and a single silver rod as the anode for 1 minute. The wires were then chloridised by dipping in fresh 0.1 M KCl and applying 1.5 volts with the polarity reversed until the bared ends of the wires were coloured reddish black.

2.4.(6) Introduction of the internal filling solution

The sensing electrodes were prepared for use by passing a length of fine needle tubing down the lumen of the PVC tubing until it lay just behind the ceramic plug and injecting the internal filling solution of 100 mM KCl. During the filling procedure care was taken to ensure that air, displaced as a result of diffusion of the solution into the hydrophilic porous ceramic plug, was evacuated by repeated flushing down the needle tubing. The needle tubing was then removed and a chloridised silver wire inserted down the PVC tubing until it came to rest just behind the ceramic plug. The proximal end of the PVC tubing was sealed with silicone rubber and the protruding silver wire soldered onto a length of insulated copper wire connecting it to an electrical connector. The area of the soldered joint was encased in plastic tubing to protect the fragile silver wire (figure 4,a).

2.4.(7) Construction of the external reference electrode

A 1 ml Gillette syringe was sectioned, leaving approximately 4 cm of the distal end of the barrel remaining. A silver/silver chloride pellet electrode (Clark Electromedical) was inserted into

the lumen and the proximal end sealed with silicone rubber. The end of the silver electrode was connected to an electrical connector by insulated copper wire, with the area of the soldered joint again encased in protective plastic tubing. The lumen was then filled with physiological saline and the modified syringe attached to a polythene cannula via a 3-way tap, also filled with saline (figure 4,b).

2.4.(8) Measurement of electrode potential

The potential difference generated between the internal and external reference electrodes was fed into a medium gain d.c. preamplifier (Gould, 13-4615-10) and displayed on a thermal writing recorder (Gould 3000S) after passing through an optical isolation circuit. The circuit used was a modification of that designed by Band, Fry and Wolf (1986). This enabled the electrical isolation of the subject and reduced electrical interference (for circuit diagram see figure 5).

The internal and external reference electrodes were plugged into a battery powered encoder unit (figure 5,a) which emitted pulses whose frequency was proportional to the size of the input voltage. The encoder output was then converted back to voltage by a separately housed decoder unit (figure 5,b). After filtering and gain adjustment in the final stage of the decoder, the signal was transfered to the Gould preamplifier/recorder assembly which produced a permanent trace.

The recorder was calibrated (in millivolt terms) by using a precision voltage source (Microcal 1030, Time Electronics) to

generate the input signals for the encoder/decoder assembly. The electrical potentials (mV) indicated on the trace during experimental recordings were converted to concentration values (mM) using calibration curves constructed for the electrodes (see section 2.4.(9)).

2.4.(9) Calibration of electrodes

The electrodes were calibrated in concentration terms in vitro using potassium chloride standards made up in a background of physiological saline (NaCl, 150 mM). This has the effect of keeping the activity coefficient of potassium at about the same value in all solutions due to the excess of sodium chloride. The temperature of the standard solutions was maintained at 37.5^{o}C, the same as the experimental environment, using a thermostated water bath.

After calibrating the recorder in voltage terms, as described in section 2.4.(8), both the ion-selective electrode and the catheter tip of the external reference electrode were immersed into standard solutions of progressively increasing KCl concentration (1-16 mM) and the potentials generated recorded. The electrode potential could then be plotted against the base 10 logarithm of the potassium concentration, producing a calibration curve (figure 6). Only electrodes that produced at least 95% of the theoretical Nernstian response were used.

The voltage range likely to be encountered during experimental recording, corresponding to a concentration range of roughly 3-7 mM potassium, could then be noted from the calibration curve and the recording system re-calibrated accordingly to cover that range at a higher sensitivity. The electrode potentials recorded during

experiments were converted to concentration values using the calibration curves.

2.4.(10) Experimental procedure

The rat was anaesthetized and the trachea and left common carotid artery cannulated as described in section 2.1.(1). The right jugular vein was isolated and the ion-selective electrode inserted into it. The electrode was advanced an appropriate length down the jugular vein until its tip rested in the vena cava at the level of the heart. The saline filled catheter tip of the external reference electrode was inserted into the left femoral vein and advanced up to the inferior vena cava. Arterial blood pressure from the carotid artery, heart rate (derived from the pulse by the Gould ECG/biotach amplifier) and electrode potential were continuously recorded on a 3-channel Gould 3000S thermal writing recorder.

Drugs were administered intravenously via the 3-way tap in the external reference electrode assembly. Animals were first given saline, followed by cumulative doses of the drug under investigation, allowing at least 30 minutes between injections. In early experiments, a single dose of adrenaline (250 ng) was administered at the beginning, to test the response of the electrode system to changes in plasma potassium concentration.

2.4.(11) Evaluation of electrodes

The accuracy and reliability of the electrode recordings were tested by taking blood samples from 15 animals at the end of the experiment, after noting the electrode potential at the time of

sampling. The plasma potassium concentrations were then determined by flame photometry, as described in section 2.3, and compared with the concentration values calculated from the electrode potentials.

2.5. The effects of desipramine on responses to tyramine

These experiments were conducted to compare the degree of neuronal uptake blockade produced by the doses of desipramine used in this study. Rats were anaesthetized and the trachea, left common carotid artery and left femoral vein cannulated, as described in section 2.1.(1). Dose-response curves for the indirectly acting sympathomimetic tyramine were constructed by noting the changes in mean arterial pressure and heart rate produced by increasing doses of tyramine (n=9). Blood pressure and heart rate were allowed to return to basal levels after each dose.

The dose-response curves were then re-constructed, starting 5 minutes after the intravenous administration of saline (n=3) or desipramine at doses of 0.1 mg/kg (n=3) and 0.5 mg/kg (n=3). Any shift in the position of the curves was noted.

2.6. Vitamin E deficient rats

Coronary occlusion experiments were carried out using a group of rats which had been fed on a vitamin E deficient diet for 8 weeks as part of a separate study, investigating the effects of vitamin E depletion on lipid peroxidation induced by myocardial ischaemia in an *in vitro* model (Zakaria, 1985). The object was to investigate any change in their vulnerability to ischaemia-induced arrhythmias *in vivo*. The experimental procedure was as described in section 2.1. and the results were compared with the control group fed on a normal diet.

2.7. Statistical analysis of data

Results are expressed as the mean ± standard error of the mean (s.e. mean).

Changes in blood pressure, heart rate and venous potassium concentration with time, following drug administration, were evaluated using the paired t-test. Means were compared with control values using the unpaired t-test or the Mann-Whitney test, as appropriate.

Statistical differences between incidences of ventricular tachycardia, ventricular fibrillation and mortality were assesed by Chi-square analysis.

Regression lines were fitted by least squares analysis.

In all cases $P < 0.05$ was considered statistically significant.

2.8. Drugs used

Drug	Manufacturer
Adrenaline hydrogen tartrate	BDH
Desipramine hydrochloride	Geigy
Idazoxan hydrochloride	Reckitt & Colman
Noradrenaline bitartrate	Sigma
DL-Propranolol hydrochloride	Sigma
Tyramine hydrochloride	Sigma
Yohimbine hydrochloride	Sigma

Drugs for intravenous administration were dissolved in physiological saline and freshly prepared each day. All doses quoted in the text refer to the salt.

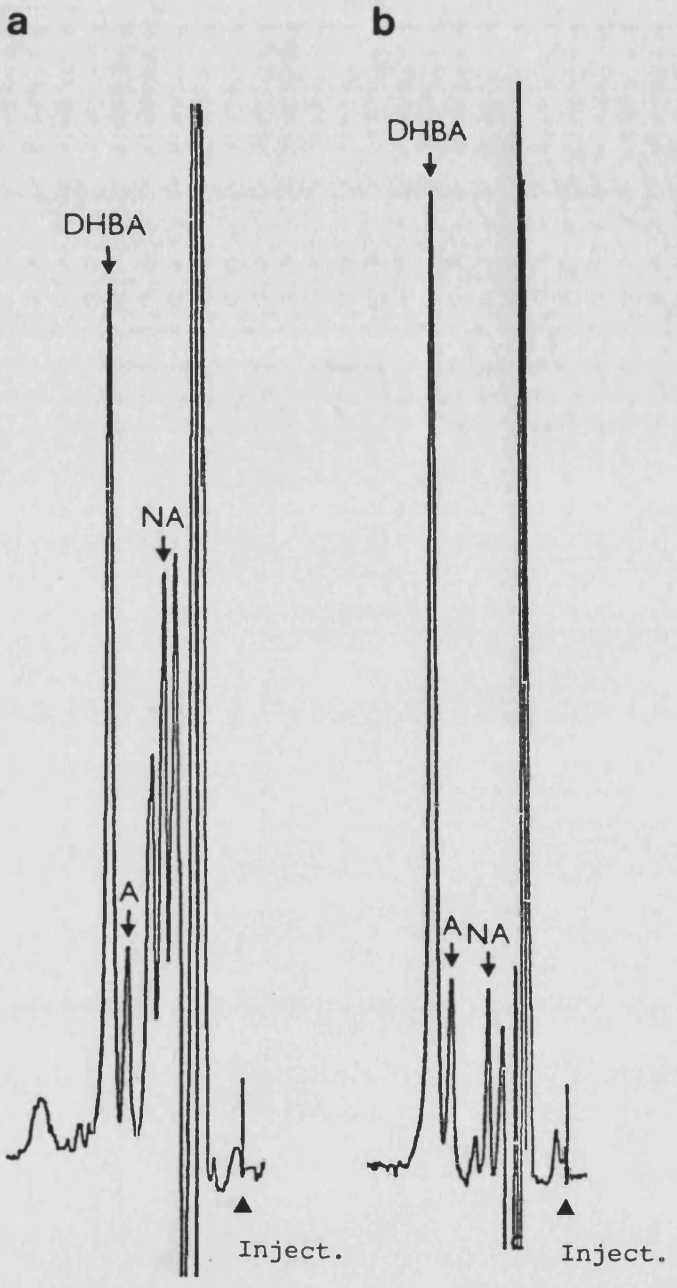


Figure 2 Typical chromatograms obtained from (a) a plasma extract, and (b) a standard solution. NA= noradrenaline, A= adrenaline, DHBA= 3,4-dihydroxybenzylamine.

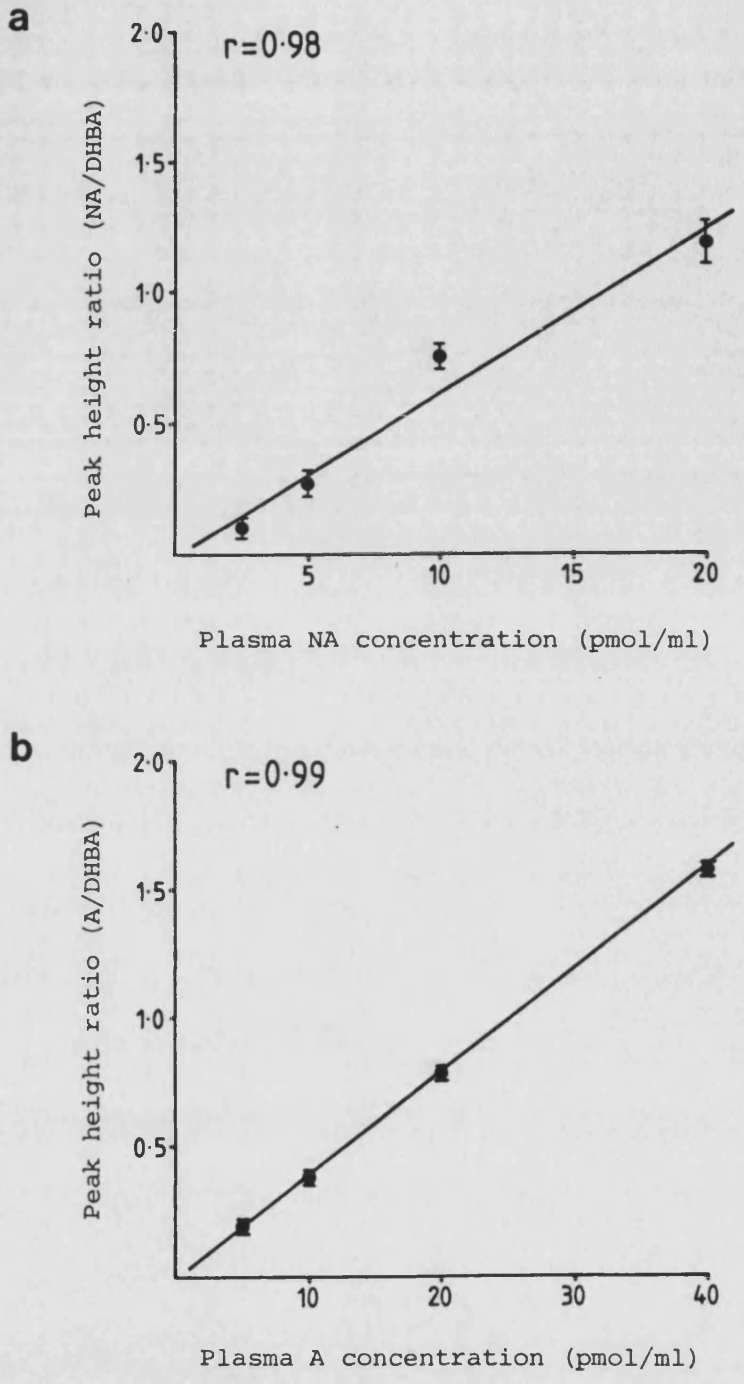


Figure 3 Calibration curves for (a) noradrenaline, and (b) adrenaline. NA=noradrenaline, A=adrenaline, DHBA= 3,4-dihydroxybenzylamine. Each point is the mean of 3 determinations and vertical lines represent the standard error of the mean.

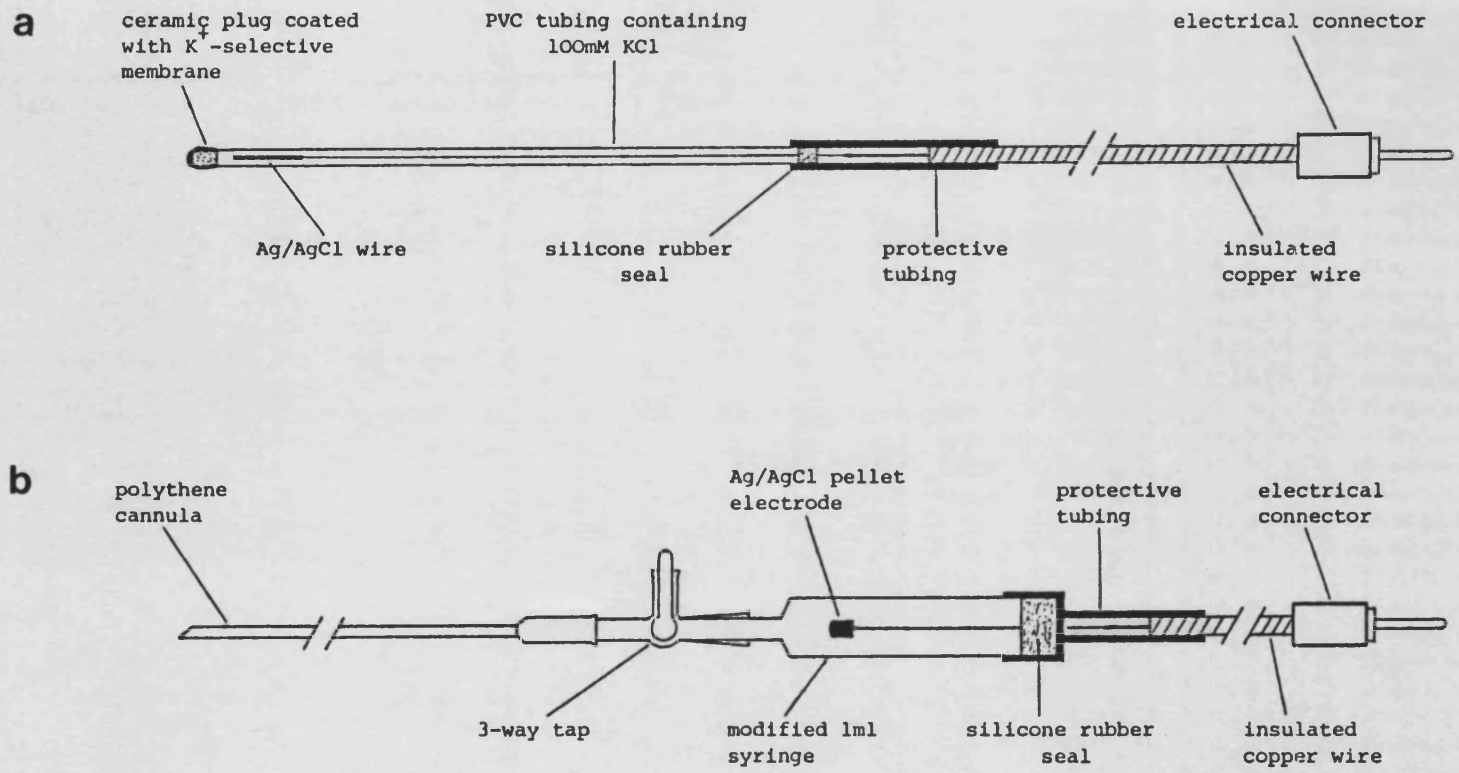


Figure 4 Structural diagrams of (a) the potassium-selective electrode, and (b) the external reference electrode.

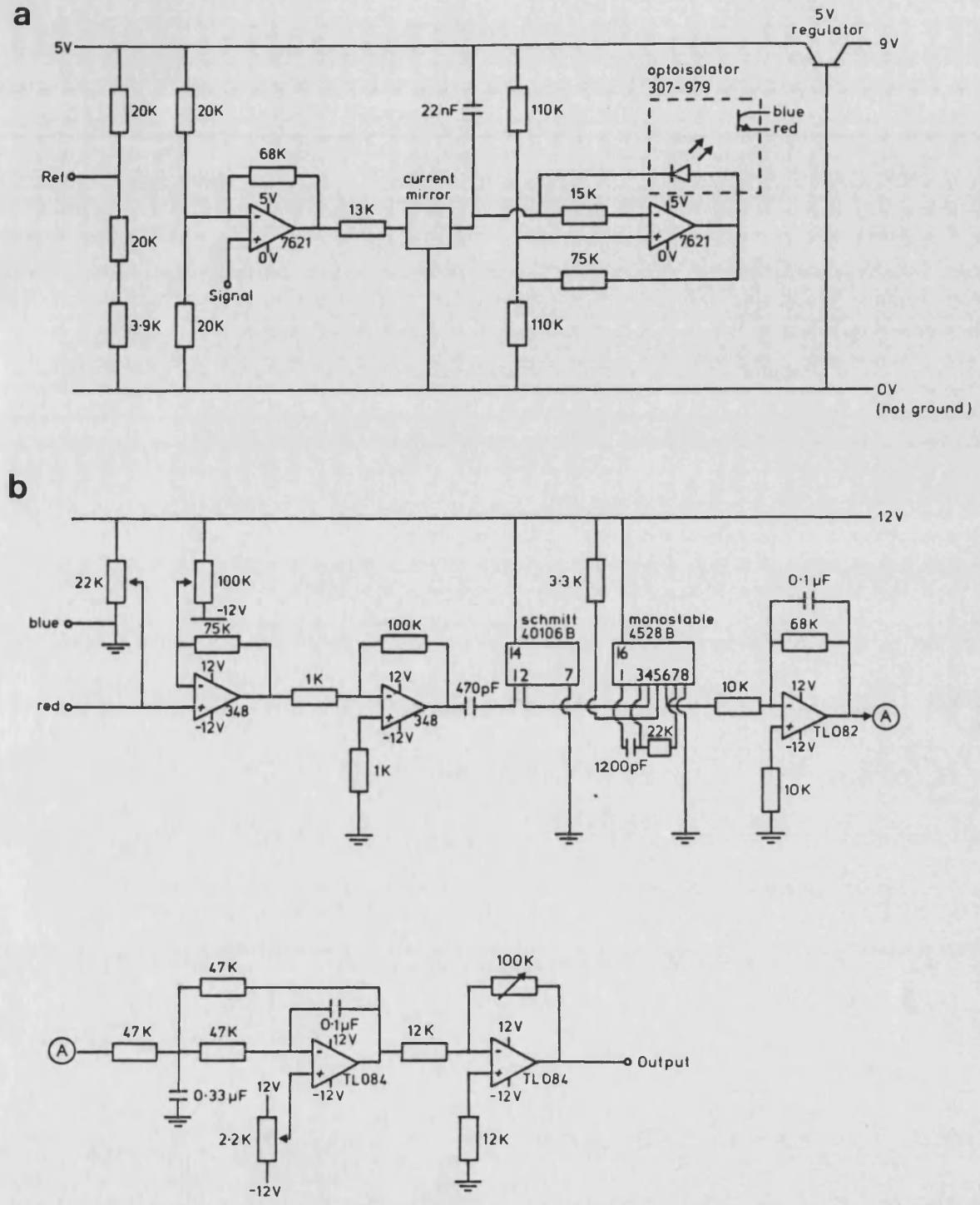


Figure 5 Circuit diagrams of (a) the encoder and (b) the decoder units of the optical isolation assembly used in conjunction with the potassium-selective electrode system. Ref=input from the external reference electrode, Signal=input from the sensing electrode.

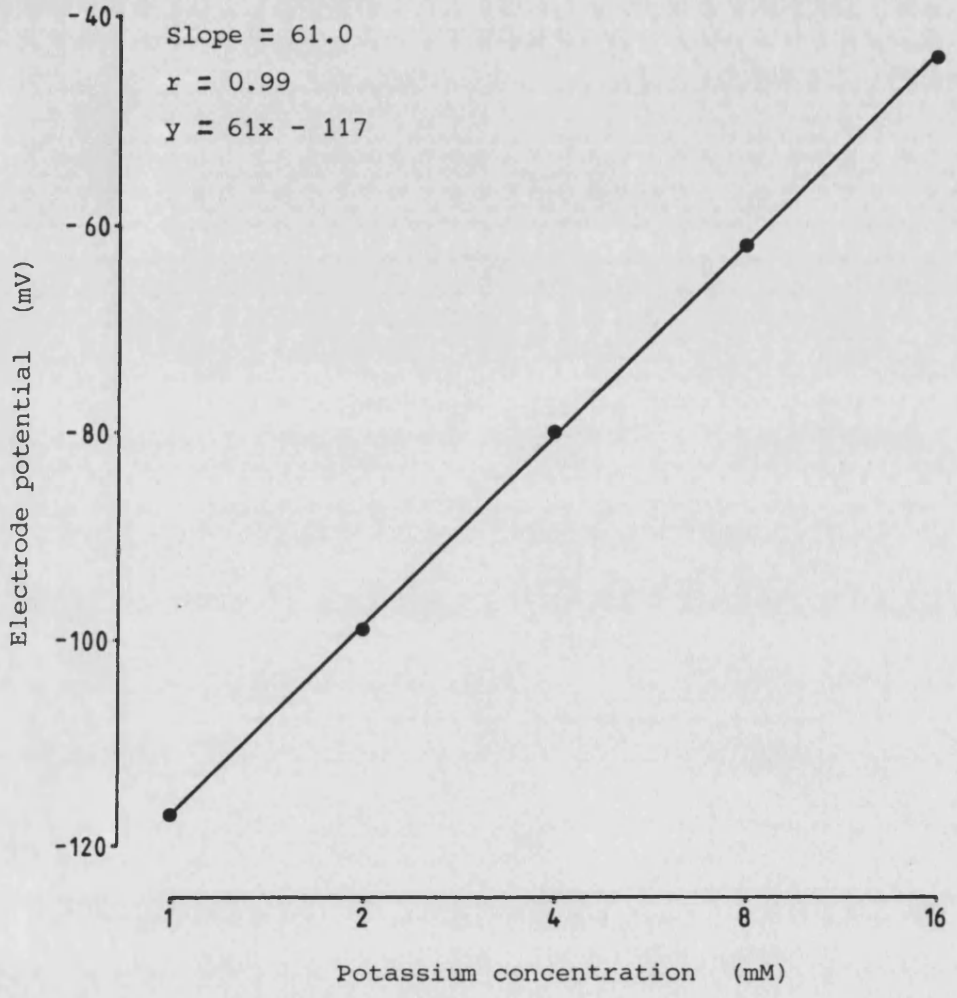


Figure 6 Typical calibration curve for a potassium-selective electrode. The theoretical Nernstian slope at 37.5 °c is 61.6 mV per decade change in potassium concentration.

Chapter 3

HAEMODYNAMIC OBSERVATIONS

3.1. Effects of coronary occlusion on blood pressure and heart rate

In control and sham occluded animals, intravenous administration of saline produced a small, transient increase in mean arterial pressure (MAP), which returned to the basal level within 3 minutes of injection. There was no effect on heart rate (HR) in either group. Consequently, at the time of coronary occlusion or sham occlusion the MAP and HR values in the two groups were very similar and not significantly different from their respective pre-saline values (Figure 7).

Following coronary occlusion there was a significant fall in MAP in the control group, from 101 $\pm$ 3 to 74 $\pm$ 2 mmHg 5 minutes after occlusion. Sham occlusion did not have any effect on MAP. Therefore, mean MAP was significantly higher in the sham occluded group than in the control group following "occlusion". Coronary occlusion or sham occlusion did not produce any change in HR and the mean values for the two groups remained similar (Figure 7).

3.2. Effects of desipramine on blood pressure and heart rate

Three doses of the neuronal uptake blocking drug desipramine (DMI) were used in this study: 0.1, 0.5 and 2.5 mg/kg i.v.. Intravenous administration of saline in the control group produced only a transient increase in MAP and did not affect HR, as mentioned in section 3.1.. The 0.1 mg/kg dose of DMI produced significant increases in both MAP and HR. The increase in MAP reached a maximum of + 28 $\pm$ 3 mmHg 2 minutes after injection and measured + 18 $\pm$ 4 mmHg 5 minutes after injection, at the time of coronary occlusion. The increase in HR produced by this dose was at its peak at the time of occlusion and measured + 67 $\pm$ 5 beats/min. Increasing the dose of

DMI to 0.5 mg/kg produced qualitatively similar responses but the increases in MAP and HR were smaller. At 2.5 mg/kg, DMI caused a sudden but short lasting reduction in MAP, measuring - 23 ± 5 mmHg 1 minute after injection, and a slower reduction in HR, reaching - 22 ± 8 beats/min by the time of coronary occlusion (Figure 8).

Figure 9 shows that at the time of drug administration mean MAP and HR values were similar in all four groups. Although MAP was higher than controls in rats treated with 0.1 and 0.5 mg/kg DMI at the time of coronary occlusion, as a result of the pressor effects of these doses, the difference was not statistically significant. Coronary occlusion produced similar reductions in MAP in all groups and there was no significant difference in mean MAP between them 5 minutes after occlusion. Groups treated with 0.1 and 0.5 mg/kg DMI had significantly higher mean HR than controls at the time of coronary occlusion. At this point, the mean HR was 479 ± 5 beats/min in the control group and 572 ± 10 and 520 ± 16 beats/min in the two DMI groups, receiving 0.1 and 0.5 mg/kg respectively. Coronary occlusion did not affect HR in any of the groups and the significant difference between the two DMI groups and controls was still evident 5 minutes after coronary occlusion. The MAP and HR values in the group receiving 2.5 mg/kg DMI were similar to control values at all three times points (Figure 9).

3.3. Effects of desipramine on the blood pressure and heart rate responses to tyramine

The effects of 0.1 and 0.5 mg/kg DMI on the responses to the indirectly acting sympathomimetic tyramine were investigated in order

to compare the degree of neuronal uptake blockade produced by these doses. The 2.5 mg/kg dose of DMI was not used because the haemodynamic effects of this dose, presented in section 3.2., suggested that it was having additional effects other than neuronal uptake blockade, such as direct myocardial depression.

Tyramine produced dose related increases in mean arterial pressure (MAP). The dose of tyramine was not increased until maximum response was obtained to avoid tachyphylaxis. There was no change in the position of the dose-response curve when it was repeated following intravenous administration of saline. Treatment with 0.1 mg/kg DMI, however, shifted the dose-respnse curve of tyramine to the right. This shift was further exaggerated by increasing the dose of DMI to 0.5 mg/kg (Figure 10). From these curves, the dose of tyramine required to produce a 10 mmHg increase in MAP was 18 μg/kg before treatment and 15, 56 and 96 μg/kg after treatment with saline, 0.1 mg/kg DMI and 0.5 mg/kg DMI respectively.

Tyramine also had a dose related positive chronotropic effect. Intravenous administration of saline had no effect on the dose-response curve but 0.1 mg/kg DMI produced a shift to the right. Increasing the dose of DMI to 0.5 mg/kg did not appear to have any further effect on the position of the dose-response curve of tyramine (Figure 11). The dose of tyramine required to produce a 50 beats/minute increase in heart rate was 43 μg/kg before treatment and 44, 115 and 110 μg/kg after treatment with saline, 0.1 mg/kg DMI and 0.5 mg/kg DMI respectively.

3.4. Effects of yohimbine, alone and in combination with desipramine, on blood pressure and heart rate

Intravenous administration of 0.1 mg/kg yohimbine, an α-adrenergic antagonist with selectivity for α_2-receptors, produced a rapid reduction in MAP which returned to the basal level within 3 minutes of injection. 1.0 mg/kg yohimbine produced a greater reduction in MAP which reached a maximum of - 27 $\pm$ 4 mmHg within 1 minute of injection and was maintained at - 17 $\pm$ 5 mmHg at the time of coronary occlusion. The lower dose of yohimbine had no effect on HR but at 1.0 mg/kg it caused a significant reduction in heart rate. This reduction in HR measured - 51 $\pm$ 7 beats/min at 1 minute following injection and returned to the basal level by the time of coronary occlusion (Figure 12).

Concomitant administration of 0.1 mg/kg DMI with these doses of yohimbine produced significant increases in both MAP and HR (Figure 12). The combination of DMI with 0.1 and 1.0 mg/kg yohimbine produced increases in MAP measuring + 25 $\pm$ 4 and + 27 $\pm$ 5 mmHg respectively 5 minutes after administration. These increases were rather similar to that obtained with DMI alone at that dose (see Figure 8). The increases in HR produced by the concomitant administration of 0.1 mg/kg DMI with 0.1 and 1.0 mg/kg yohimbine were, however, greater than that caused by DMI alone, measuring + 83 $\pm$ 5 and + 115 $\pm$ 8 beats/min respectively 5 minutes after administration. The increases in MAP and HR produced by the combination of DMI with the higher dose of yohimbine were slower to take effect due to the depressor and negative chronotropic effects observed with yohimbine at that dose.

Mean MAP and HR values were similar in all groups prior to drug

administration (Figure 13). At the time of coronary occlusion, the group treated with the 0.1/0.1 mg/kg combination of yohimbine/DMI had a mean MAP of 127 ± 4 mmHg which was significantly higher than the control mean MAP of 101 ± 3 mmHg. The group receiving the combination of DMI with 1.0 mg/kg yohimbine also had a higher mean MAP (117 ± 6 mmHg) than control at this time point, but the difference was not statistically significant. Of the groups receiving yohimbine alone, mean MAP was similar to control in the 0.1 mg/kg group but significantly lower at 78 ± 5 mmHg in the 1.0 mg/kg group. Following coronary occlusion, the mean MAP remained significantly higher than control in the group receiving 0.1/0.1 mg/kg yohimbine/DMI. The values for the other three groups did not significantly differ from control 5 minutes after coronary occlusion (Figure 13, a).

Both groups receiving combinations of yohimbine and desipramine had significantly higher mean HR than control at the time of coronary occlusion (Figure 13, b). The mean HR in the groups given 0.1/0.1 and 1.0/0.1 mg/kg combinations of yohimbine/DMI were 528 ± 10 and 598 ± 8 beats/min respectively at this time point, compared with the control value of 479 ± 5 beats/min. As coronary occlusion did not affect HR in any of the groups, the significant difference in mean HR between the two yohimbine/DMI groups and control remained 5 minutes after occlusion. The mean HR was not significantly different from control in either of the groups treated with yohimbine alone (0.1 and 1.0 mg/kg) at any time point.

3.5. Effects of idazoxan, alone and in combination with desipramine, on blood pressure and heart rate

Idazoxan, another α_2-adrenoceptor antagonist, caused small but significant increases in both MAP and HR at 0.03 mg/kg. The 0.3 mg/kg dose of idazoxan produced larger increases in MAP and HR, measuring + 30 ± 4 mmHg and + 36 ± 7 beats/min respectively five minutes after administration. The combination of 0.3 mg/kg idazoxan with 0.1 mg/kg DMI caused very large increases in both parameters. The mean increases in MAP and HR produced by this combination 5 minutes after administration were + 60 ± 6 mmHg and + 114 ± 9 beats/min respectively (Figure 14).

Prior to drug administration the mean MAP and HR values in the treatment groups were not significantly different from control, although the group due to receive 0.03 mg/kg idazoxan had a slightly higher mean MAP. As a result of the pressor effects of both doses of idazoxan and the idazoxan/DMI combination, the mean MAP values in the three treatment groups were significantly higher than control at the time of coronary occlusion. The mean MAP values for the groups receiving 0.03 and 0.3 mg/kg idazoxan and the 0.3/0.1 mg/kg combination of idazoxan/DMI were 128 ± 6, 125 ± 5 and 151 ± 7 mmHg respectively, compared to 101 ± 3 mmHg in the control group. Coronary occlusion produced similar reductions in MAP in all groups and the significant difference in mean MAP between the treatment and control groups remained 5 minutes after occlusion (Figure 15, a).

All groups had similar mean HR values before drug administration but the group receiving the combination of idazoxan and DMI had a significantly higher mean HR than control both at the time of coronary occlusion (564 ± 10 versus 479 ± 5 beats/min in control),

and 5 minutes after coronary occlusion (570 ± 9 versus 469 ± 6 beats/min in control). The mean HR values in the two groups receiving idazoxan alone were not significantly different from control at either time point (Figure 15,b).

3.6. Effects of coronary occlusion on blood pressure and heart rate in vitamin E deficient rats

Rats fed on a vitamin E deficient diet for 8 weeks had a mean serum vitamin E concentration of 11.0 ± 0.6 mM compared with 20.1 ± 0.7 mM in control rats (n=9), as measured by Zakaria (1985). The vitamin E deficient group had similar mean MAP and HR values to the control group, which had received a normal diet, prior to intravenous administration of saline and at the time of coronary occlusion. Following coronary occlusion, however, the vitamin E deficient group had a slightly higher mean MAP and a significantly higher mean HR than the control group. The mean HR values 5 minutes after coronary occlusion in the vitamin E deficient and control groups were 507 ± 10 and 469 ± 6 beats/min respectively (Figure 16).

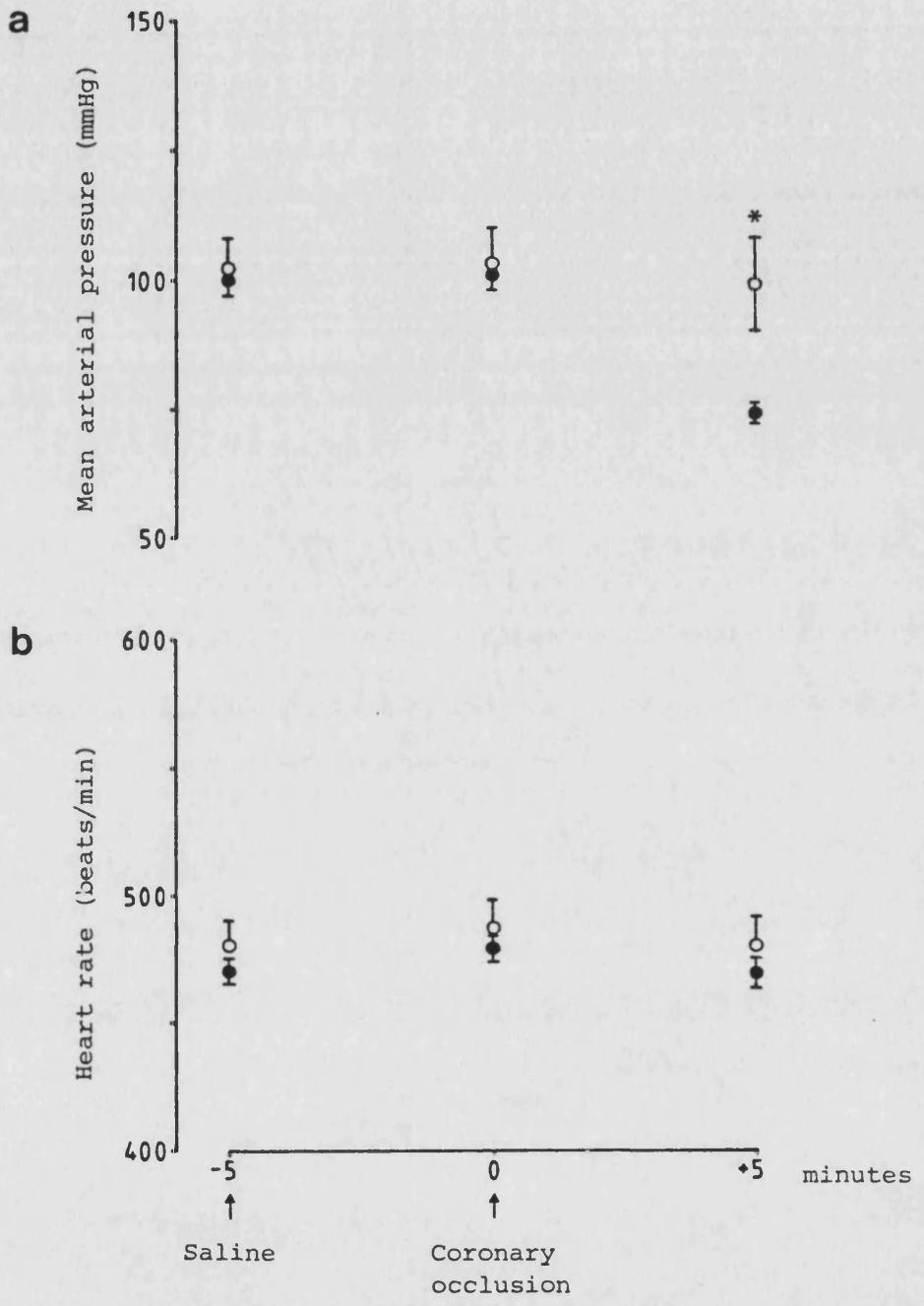


Figure 7 Effects of coronary occlusion in controls (●,n=73) and sham occlusion (O,n=10) on (a) mean arterial pressure and (b) heart rate, following intravenous administration of saline. All values are means and vertical lines represent s.e.mean. * denotes significant difference from control ($P<0.05$).

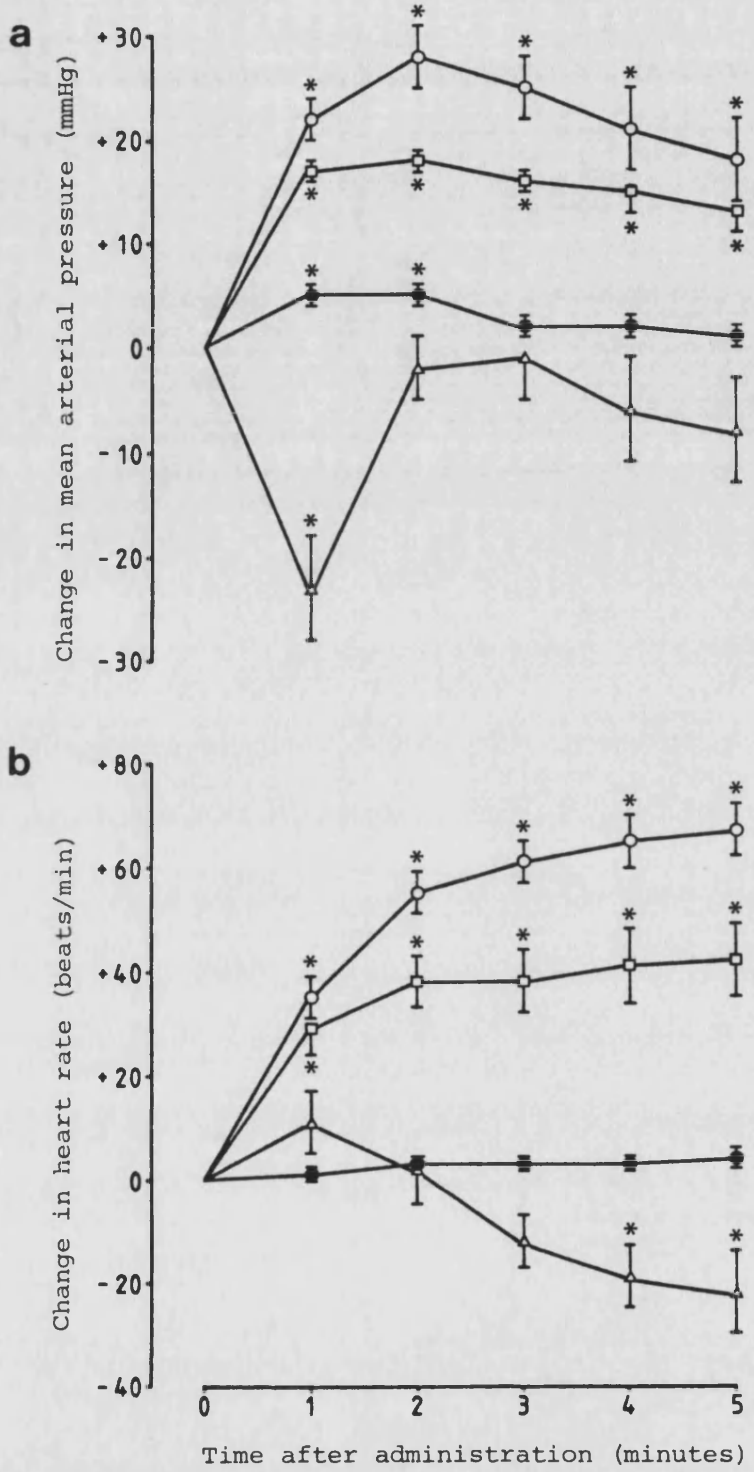


Figure 8 Effects of intravenous administration of saline in controls (●,n=73) and desipramine at doses of 0.1 (○,n=12), 0.5 (□, n=12) and 2.5 (△,n=13) mg/kg on (a) mean arterial pressure and (b) heart rate. All values are means and vertical lines represent s.e.mean. * denotes significant difference from 0 minute value ($P<0.05$).

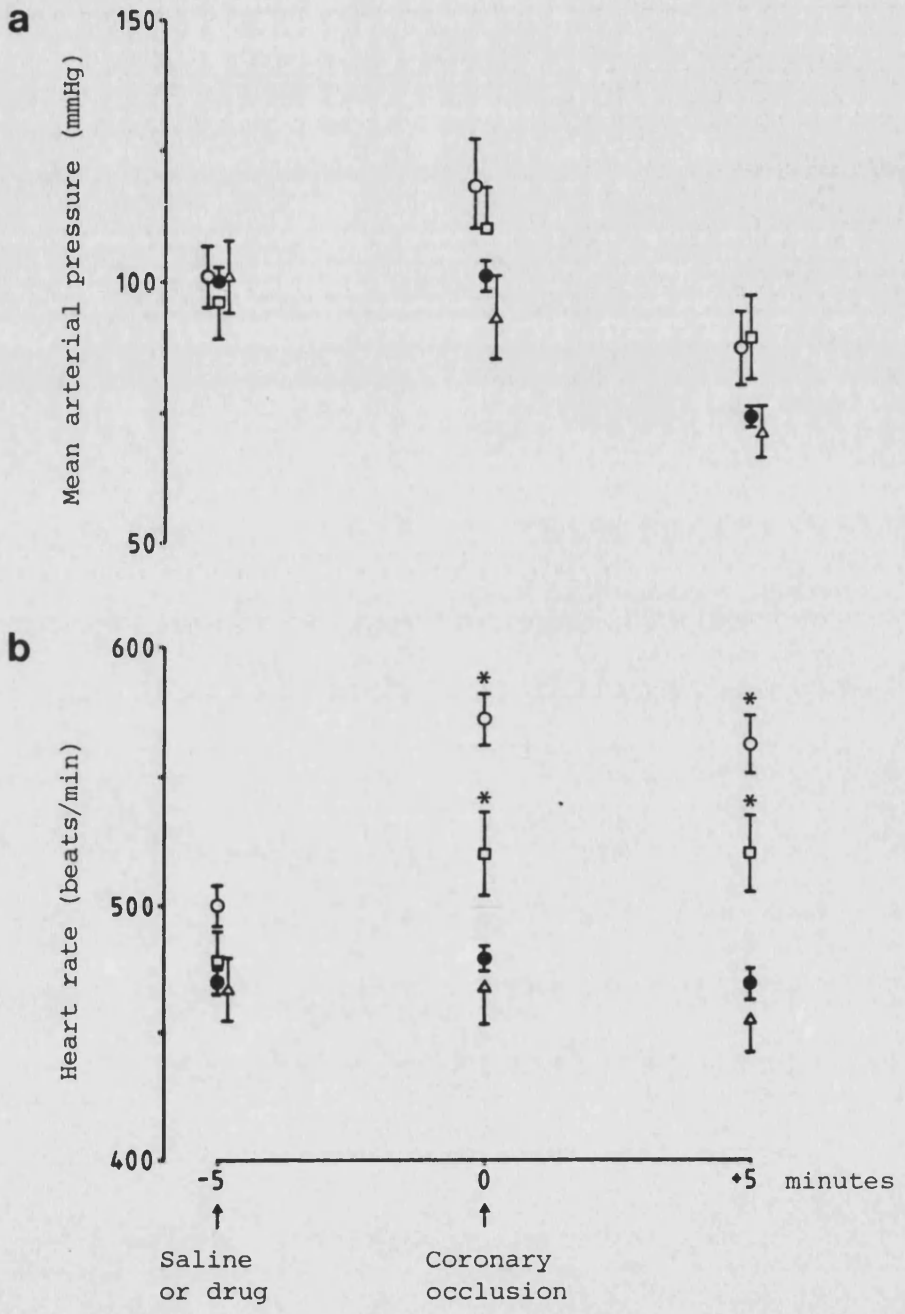


Figure 9 Effects of coronary occlusion on (a) mean arterial pressure and (b) heart rate following intravenous administration of saline in controls (●,n=73) and desipramine at doses of 0.1 (○,n=12), 0.5 (□,n=12) and 2.5 (△,n=13) mg/kg. All values are means and vertical lines represent s.e.mean. * denotes significant difference from control ($P<0.05$).

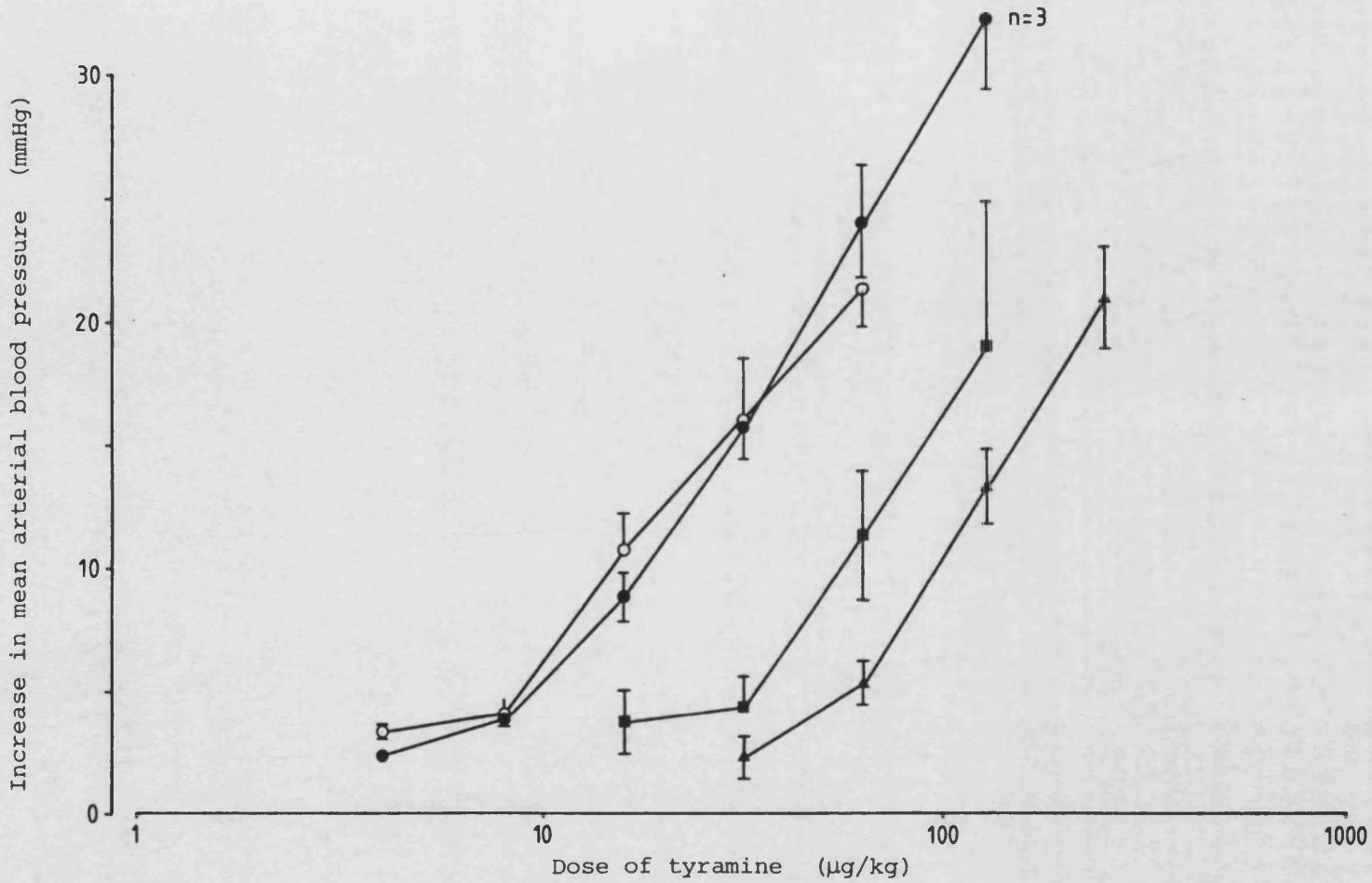


Figure 10 Effects of tyramine on mean arterial pressure before treatment (●,n=9) and after intravenous administration of saline (O,n=3) and desipramine at doses of 0.1 (■,n=3) and 0.5 (▲,n=3) mg/kg. All values are means and vertical lines represent s.e.mean.

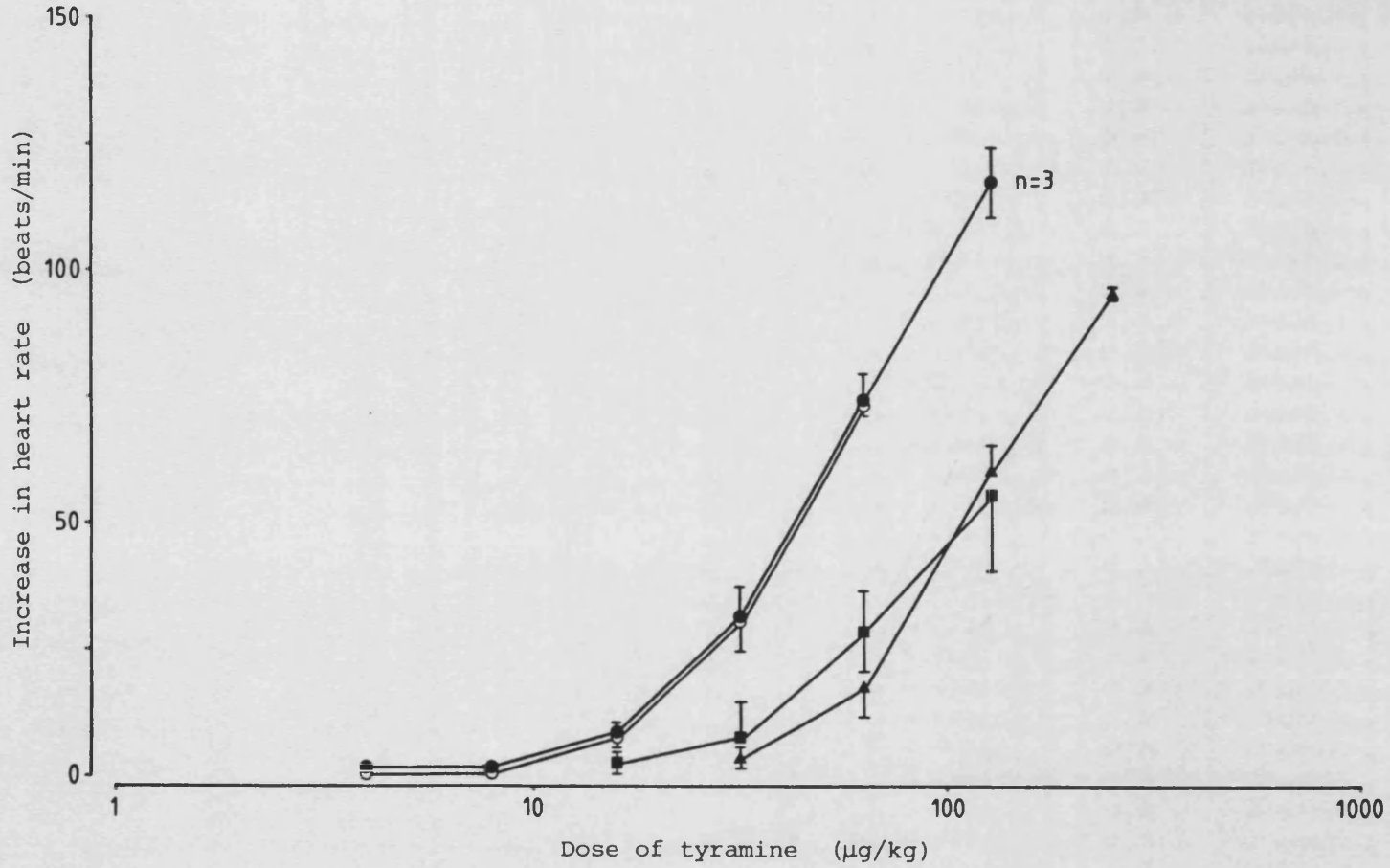


Figure 11 Effects of tyramine on heart rate before treatment (●,n=9) and after intravenous administration of saline (O,n=3) and desipramine at doses of 0.1 (■,n=3) and 0.5 (▲,n=3) mg/kg. All values are means and vertical lines represent s.e.mean.

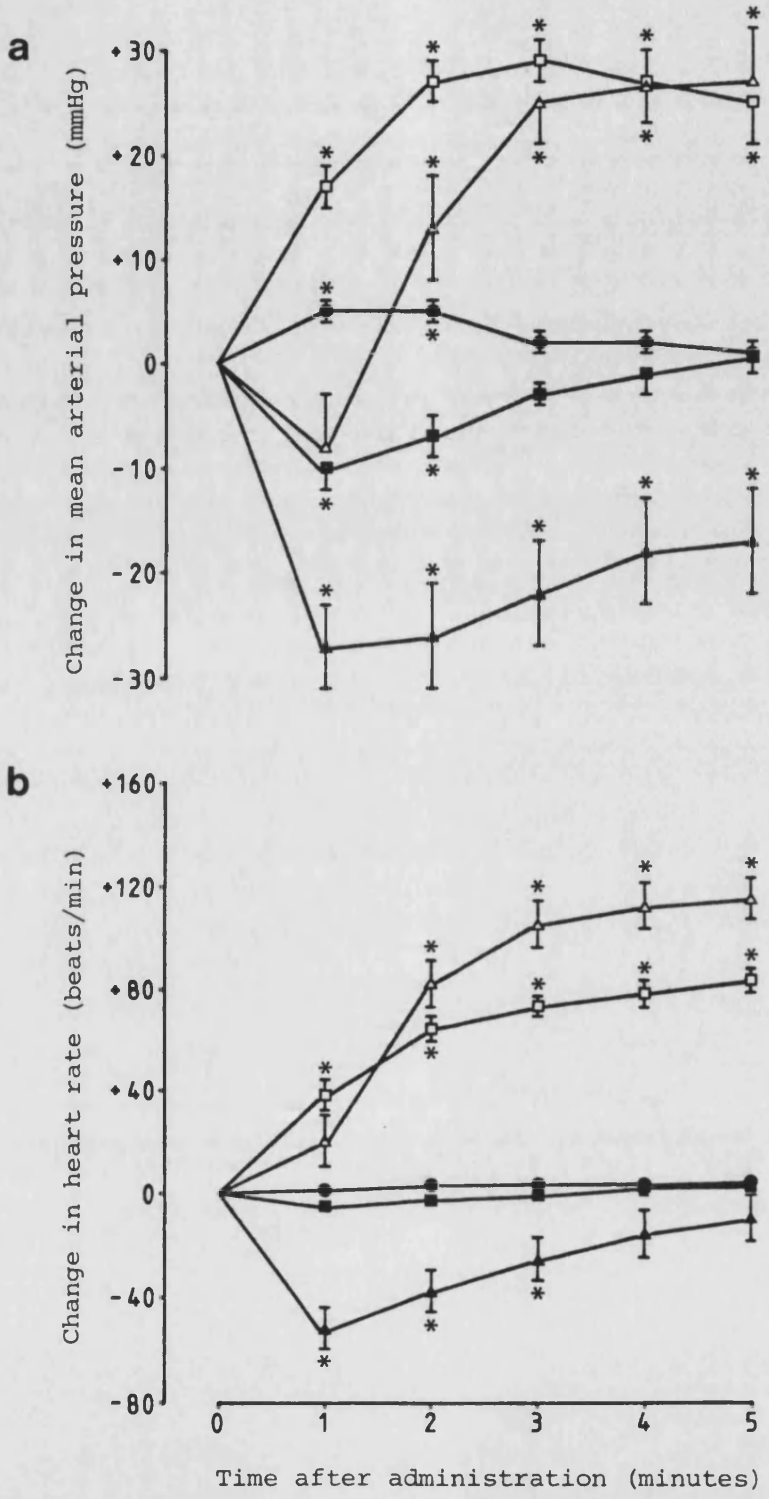


Figure 12 Effects of intravenous administration of saline in controls (●,n=73), yohimbine at doses of 0.1 (■,n=18) and 1.0 (▲,n=12) mg/kg, and combined yohimbine/desipramine at doses of 0.1/0.1 (□,n=14) and 1.0/0.1 (△,n=12) mg/kg on (a) mean arterial pressure and (b) heart rate. All values are means and vertical lines represent s.e.mean. * denotes significant difference from 0 minute value ($P<0.05$).

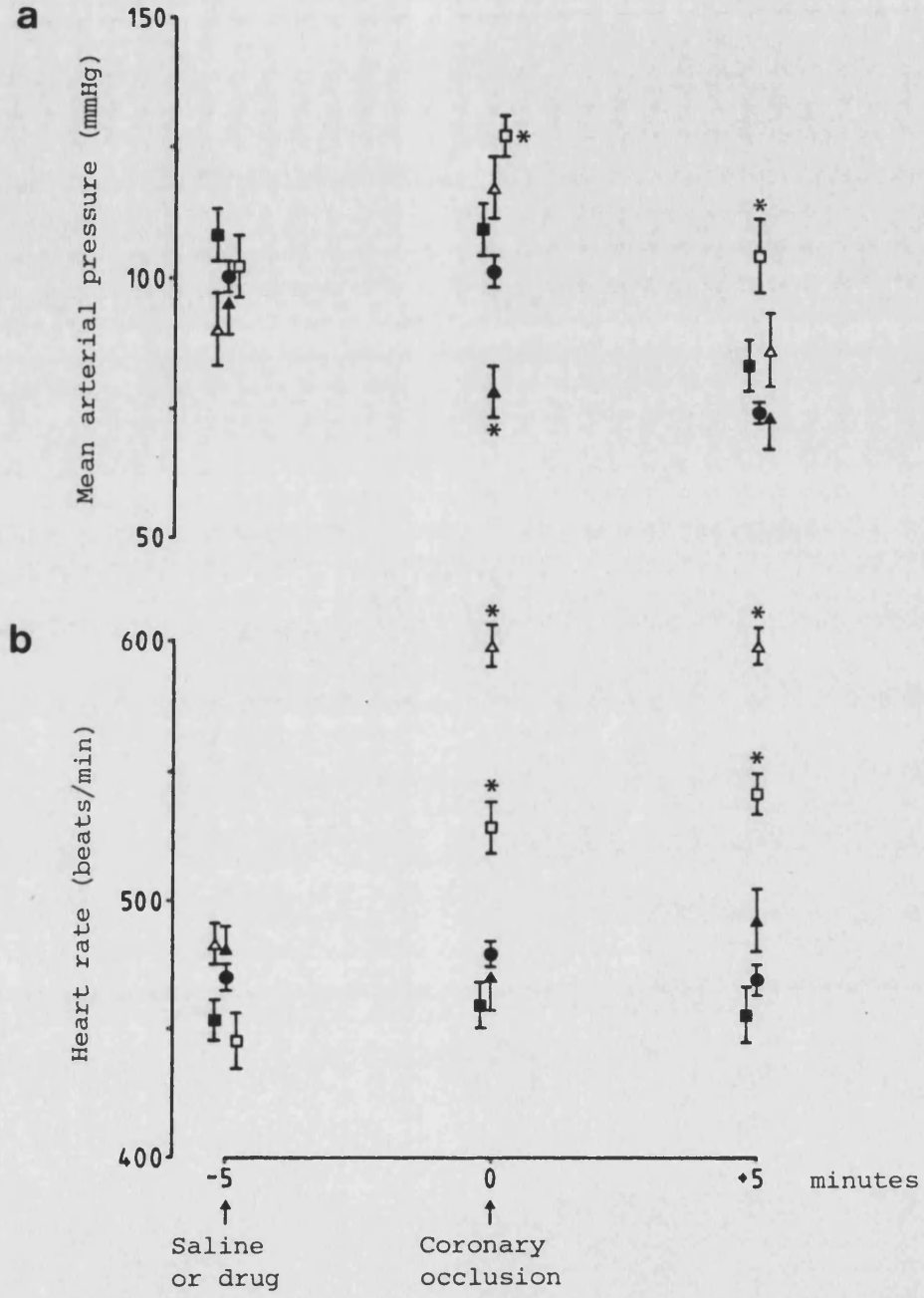


Figure 13 Effects of coronary occlusion on (a) mean arterial pressure and (b) heart rate following intravenous administration of saline in controls (●,n=73), yohimbine at doses of 0.1 (■, n=18) and 1.0 (▲,n=12) mg/kg, and combined yohimbine/ desipramine at doses of 0.1/0.1 (□,n=14) and 1.0/0.1 (△,n=12) mg/kg. All values are means and vertical lines represent s.e. mean. * denotes significant difference from control ($P<0.05$).

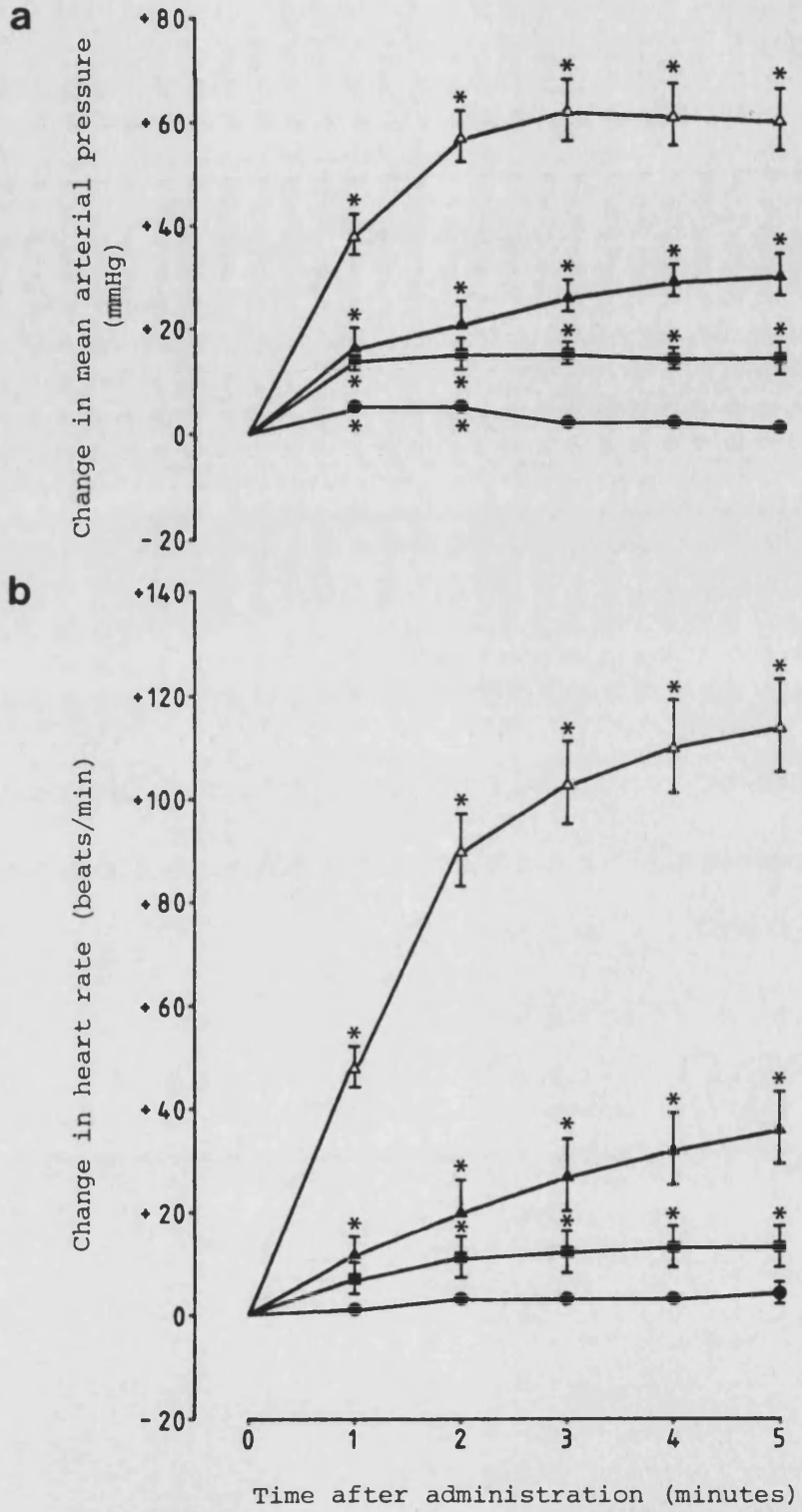


Figure 14 Effects of intravenous administration of saline in controls (●,n=73), idazoxan at doses of 0.03 (■,n=12) and 0.3 (▲, n=18) mg/kg, and combined idazoxan/desipramine, 0.3/0.1 mg/kg (△,n=14), on (a) mean arterial pressure and (b) heart rate. All values are means and vertical lines represent s.e.mean. * denotes significant difference from 0 minute value ($P<0.05$).

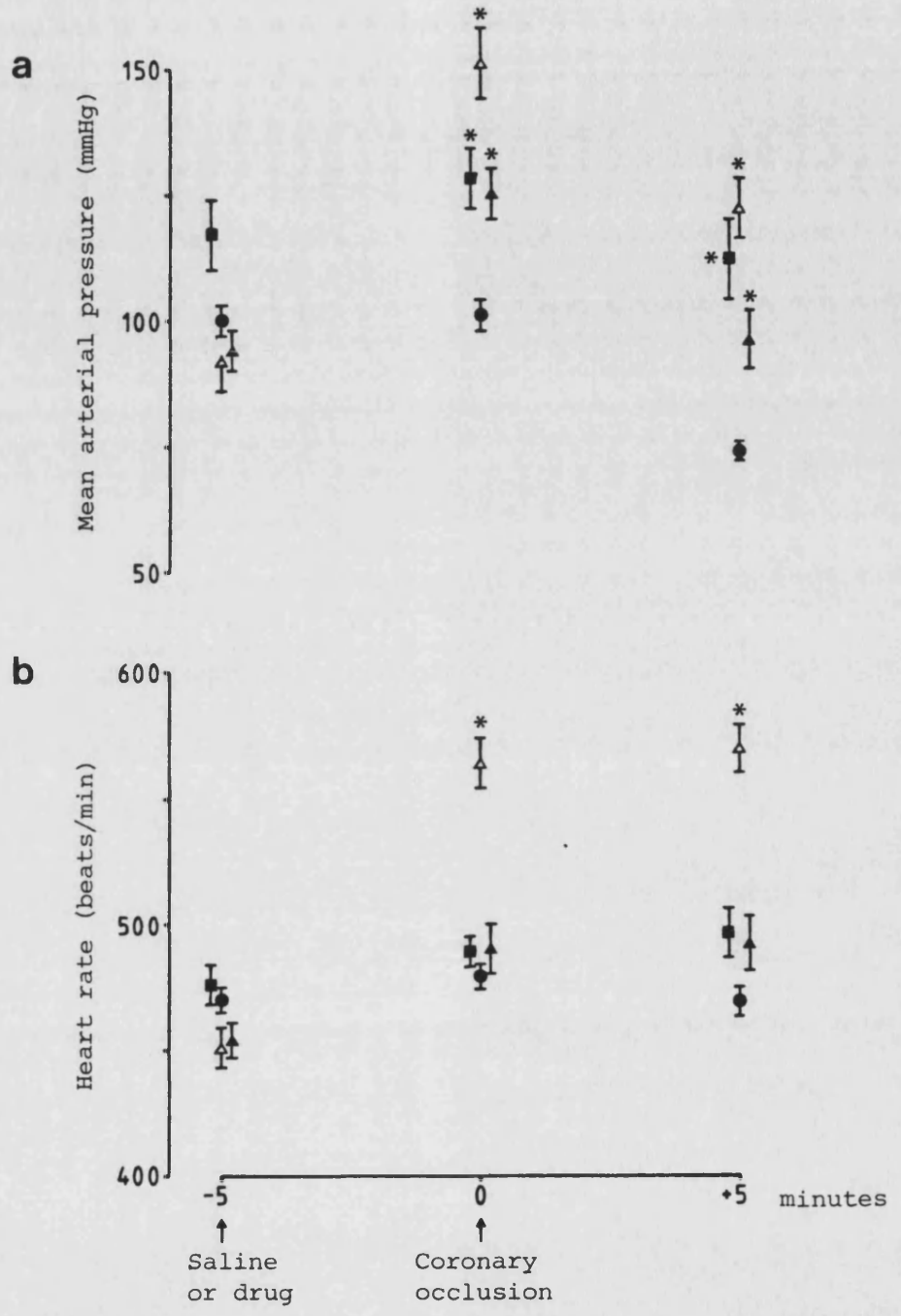


Figure 15 Effects of coronary occlusion on (a) mean arterial pressure and (b) heart rate following intravenous administration of saline in controls (●,n=73), idazoxan at doses of 0.03 (■, n=12) and 0.3 (▲,n=18) mg/kg, and combined idazoxan/ desipramine, 0.3/0.1 mg/kg (△,n=14). All values are means and vertical lines represent s.e.mean. * denotes significant difference from control ($P<0.05$).

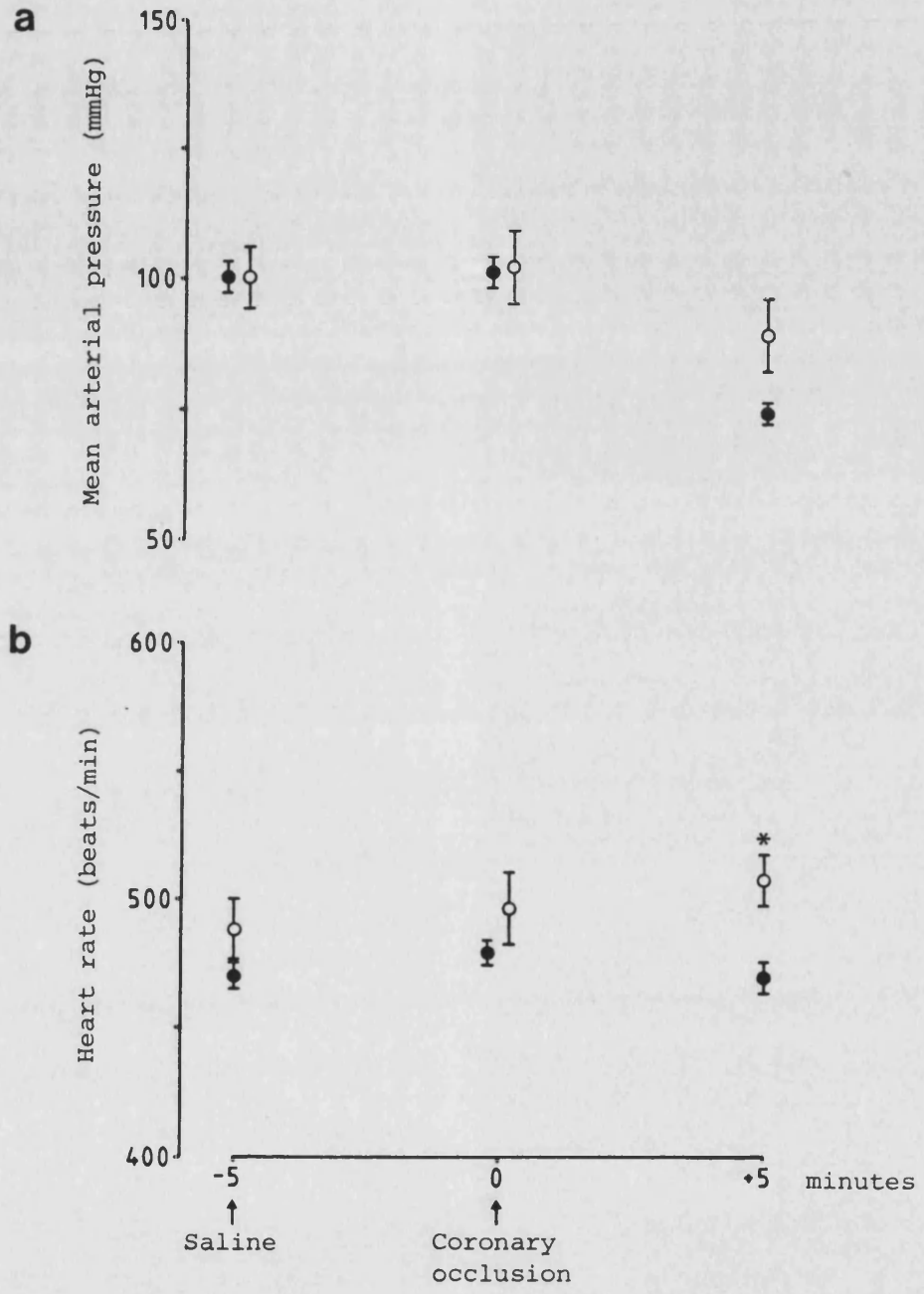


Figure 16 Effects of coronary occlusion on (a) mean arterial pressure and (b) heart rate in control (●,n=73) and vitamin E deficient (O,n=15) groups, following intravenous administration of saline. All values are means and vertical lines represent s.e.mean. * denotes significant difference from control ($P<0.05$).

Chapter 4

EFFECTS ON ARRHYTHMIAS

4.1. Coronary occlusion-induced arrhythmias

Occlusion of the left anterior descending coronary artery produced a large increase in ECG signal size, resulting from an increase in R-wave amplitude. This effect was very rapid and occurred within seconds of coronary occlusion, accompanied by a reduction in blood pressure, as discussed in section 3.1. Heart rate was not affected by coronary occlusion.

Subsequently there was a gradual reduction in R-wave amplitude prior to the development of arrhythmias, which usually started within 5 minutes of coronary occlusion. The arrhythmias usually lasted for 8-10 minutes and had subsided by 15 minutes after coronary occlusion. Following this phase of arrhythmias there was often an increase in blood pressure and heart rate in surviving animals, perhaps as part of a reflex response to the haemodynamic fluctuations caused by the arrhythmias.

There was also a slowly developing, sustained "ST-segment" elevation which started within 2-3 minutes of coronary occlusion and continued until the end of the occlusion period (the rat ECG has no true ST-segment and the term "ST-segment" has been used here to describe the junction of the QRS and T waves). Figure 17 displays a representative trace of blood pressure, ECG and heart rate recordings from a control experiment, showing the changes that occurred in these parameters following coronary occlusion. The ST-segment elevation that had occurred after 15 minutes of coronary occlusion is also indicated.

The changes in the ECG pattern following coronary occlusion are better illustrated in Figure 18. An elevated ST-segment was evident 5 min after coronary occlusion and this was further exaggerated with

increasing duration of ischaemia. Another notable electrocardiographic change was the development of Q-waves towards the end of the occlusion period, as illustrated 20 min post-occlusion in this figure.

The various types of arrhythmias that are observed in this model are illustrated in Figure 19 which depicts a representative high speed blood pressure and lead II ECG recording from a control experiment, around 8 minutes after coronary occlusion. The arrhythmias occurred mainly between 4 and 15 minutes post-occlusion and comprised premature ventricular contractions (PVCs) and bursts of ventricular tachycardia (VT) and ventricular fibrillation (VF), which in the rat can spontaneously revert to sinus rhythm. However, persistent VT and VF, lasting several minutes, were observed in some rats and resulted in death. Sham occlusion of the coronary artery in 10 animals did not produce any arrhythmias.

4.2. Arrhythmias in control experiments

During this study five separate control groups were established between January 1984 and October 1985, with 11-21 animals in each group. The experiments in these groups, designated the letters A to E, were carried out at the following dates:

Control Group	n	Dates of experiments
A	21	January 1984
B	12	March 1984
C	11	May-June 1984
D	15	February-April 1985
E	14	September-October 1985

There was no significant seasonal variation in the incidence and severity of arrhythmias in these control experiments (Figure 20). The total number of PVCs during the 20 min coronary occlusion period ranged from 541 ± 100 in group D to 954 ± 300 in group C, with the means from the other groups falling between these two extremes. The incidences of VT and VF were similar in all groups at around 80-90% and 20-30% respectively. The greatest variability was in the incidence of mortality which ranged from 0% in group E to 24% in group A. However, even these two extremes were not significantly different from each other when compared by the Chi-square test.

Figure 21 shows the mean values obtained from the control groups for the onset of the first episode and the total duration of all episodes of both VT and VF. There was no significant difference in any of these parameters between the five groups. The distribution of PVCs during the 20 min occlusion period was also similar in all groups with the majority taking place between 4 and 15 min after coronary occlusion, although peak ectopic activity appeared to occur sooner in group D than in any of the other groups (Figure 22).

For practical purposes the data from the five groups were pooled together to form one large control group (n=73) for comparison with treatment groups. The total number of PVCs in this combined control group was 741 ± 93 and the incidences of VT, VF and mortality were 84%, 29% and 12% respectively. The mean onset of the first episode was 5.4 ± 0.3 min for VT and 7.2 ± 0.2 min for VF following coronary occlusion. The mean total duration measured 55 ± 8 s and 30 ± 4 s for VT and VF respectively.

4.3. Effects of desipramine on arrhythmias

The neuronal uptake blocking agent desipramine (DMI) had no significant effect on the arrhythmias at the 0.1 mg/kg dose which produced significant increases in blood pressure and heart rate. Increasing the dose of DMI appeared to have a dose-related antiarrhythmic effect, significantly reducing the incidence of VT from 84% in controls to 42% at 0.5 mg/kg and 31% at 2.5 mg/kg despite the contrasting effects these two doses had on blood pressure and heart rate. These doses of DMI also abolished the incidence of VF and there was no mortality in either group. The total number of PVCs was significantly reduced by 0.5 mg/kg DMI, from 741 $\pm$ 93 in the control group to 182 $\pm$ 48, but not affected by the 2.5 mg/kg dose (Figure 23).

The mean onset and duration of VT in groups treated with 0.1 and 2.5 mg/kg doses of DMI were not significantly different from control values, although the onset of VT appeared to have been accelerated in the 4 animals that exhibited VT in the 2.5 mg/kg group. 0.5 mg/kg DMI, however, significantly reduced the duration of VT from 55$\pm$ 8 s in controls to 11 $\pm$ 5 s and this was reflected by the reduction in the number of PVCs in this group (see above). The onset of VT was not significantly affected. Of the groups receiving DMI, only the 0.1 mg/kg group exhibited VF and the onset and duration of VF were not significantly different from control values (Table 1).

The distribution of PVCs during the occlusion period was not affected by 0.1 mg/kg DMI. Although 0.5 mg/kg DMI significantly suppressed the occurrence of PVCs it did not appear to alter their distribution. 2.5 mg/kg DMI, however, caused a shift in peak ectopic activity which occurred at 5 min after coronary occlusion compared

to 8 min in the control group (Figure 24), possibly as a consequence of the accelerated onset of VT.

4.4. Effects of yohimbine, alone and in combination with desipramine, on arrhythmias

DMI, as a neuronal uptake blocker, would be expected to elevate synaptic levels of noradrenaline at sympathetic nerve terminals. In order to investigate whether such an action was involved in the anti-arrhythmic mechanism of DMI, the effects of the α_2-adrenoceptor antagonist yohimbine on arrhythmias were examined, both when given alone and in combination with DMI. Yohimbine would also be expected to elevate the synaptic levels of noradrenaline, by attenuating the presynaptic inhibition of release, and should enhance the effects of DMI when given in combination.

Yohimbine, at 0.1 mg/kg, had no lasting effects on blood pressure and heart rate but produced a significant increase in the incidence of VF from 29% in the control group to 56%. This was accompanied by a significant increase in mortality from 12% in the control group to 33%. The number of PVCs and the incidence of VT were not significantly affected by this dose of yohimbine. At the larger dose of 1.0 mg/kg, which produced a significant reduction in blood pressure and a transient reduction in heart rate, yohimbine had the opposite effect, producing significant reductions in all parameters of arrhythmic activity. The number of PVCs was reduced from 741 $\pm$ 93 in the control group to 222 $\pm$ 107, the incidence of VT from 84% to 33% and the incidence of VF from 29% to 0%. There was also no mortality in the group receiving 1.0 mg/kg yohimbine (Figure 25).

Both doses of yohimbine were also given in combination with 0.1 mg/kg DMI. Concomitant administration of DMI with 0.1 mg/kg yohimbine produced significant increases in blood pressure and heart rate and appeared to abolish the arrhythmogenic effects of this dose of yohimbine alone. The number of PVCs in this group was 629 $\pm$ 171 and the incidences of VT, VF and mortality were 79%, 21% and 7% respectively. These values were not significantly different from their respective controls. When 1.0 mg/kg yohimbine was administered in combination with DMI, there were large increases in both blood pressure and heart rate but the significant antiarrhythmic effect observed with that dose of yohimbine alone persisted. The number of PVCs was decreased to 343 $\pm$ 150 and the incidences of VT, VF and mortality were reduced to 33%, 0% and 0% respectively. These values were very similar to those observed in the group receiving yohimbine alone at 1.0 mg/kg (Figure 25).

Table 2 gives the onset and duration values for VT and VF in the control, yohimbine and combined yohimbine/DMI groups. There was no significant difference from control in any of the treatment groups, although the onset of VT appeared to be sooner in the 4 animals which exhibited VT in the group receiving the combination of 1.0 mg/kg yohimbine with DMI (4.1 $\pm$ 0.4 min compared with 5.4 $\pm$ 0.3 min in the control group).

The distribution of PVCs during the 20 min coronary occlusion period in the control and treatment groups is shown in Figure 26. At 0.1 mg/kg, yohimbine did not appear to affect the distribution of PVCs, either alone or in combination with DMI. The 1.0 mg/kg dose of yohimbine suppressed the occurrence of PVCs, both when given alone and in combination with 0.1 mg/kg DMI. Yohimbine alone at this dose

did not alter the distribution of PVCs but peak ectopic activity appeared to occur sooner in the group receiving the combination of 1.0 mg/kg yohimbine and DMI, possibly as a result of the accelerated onset of VT in the 4 animals which exhibited VT in this group.

4.5. Effects of combined post-occlusion administration of yohimbine and desipramine on arrhythmias

In order to investigate whether the antiarrhythmic effect of the combination of 1.0 mg/kg yohimbine and 0.1 mg/kg DMI was mediated by a localized action within the ischaemic myocardium or by a general sympathetic activation, a group of animals were given this combination 2 min after coronary occlusion. It was assumed that in these animals the drugs would not enter the ischaemic area in any appreciable quantity due to the low collateral flow in the rat.

Figure 27 shows the effects of pre- and post-occlusion administration of 1.0 mg/kg yohimbine in combination with 0.1 mg/kg DMI on arrhythmias. Treatment with this combination prior to coronary occlusion had a significant antiarrhythmic effect, as described in section 4.4.. Its administration 2 min after coronary occlusion produced increases in blood pressure and heart rate similar to those observed with pre-occlusion administration but had no significant effect on the occurrence and severity of arrhythmias when compared to the control group which received an injection of saline at the same time. There was an increase in mortality from 8% in controls to 33% but the difference did not reach statistical significance.

Post-occlusion administration of this combination of yohimbine and desipramine did not affect the onset and duration of VT but significantly accelerated tne onset of VF from 7.2 ± 0.3 min in controls to 5.0 ± 0.4 min without altering its duration (Table 3).

4.6. Effects of acute bilateral vagotomy on arrhythmias in rats treated with a combination of yohimbine and desipramine

The antiarrhythmic effect of the yohimbine/DMI combination may be mediated by a reflex increase in vagal activity in response to the large increases in blood presure and heart rate produced by this combination. These experiments were carried out to investigate this possibility.

Bilateral vagotomy was performed at various times after coronary occlusion in a small number of animals treated with the combination of 1.0 mg/kg yohimbine and 0.1 mg/kg DMI 5 min before occlusion. Both vagi, which had been isolated from the adjacent nerves and connective tissue around the left and right common carotid arteries, were sectioned at 3(n=4), 7(n=4) and 10 min (n=3) after coronary occlusion. Bilateral vagotomy did not appear to affect the anti-arrythmic efficacy of this combination of yohimbine and DMI and no increase in ectopic activity was observed following vagotomy in any of the groups. Figure 28 shows the lack of effect of acute bilateral vagotomy at 3,7 and 10 min after coronary occlusion on the occurence and distribution of subsequent PVCs.

4.7. Effects of idazoxan, alone and in combination with desipramine, on arrhythmias

Yohimbine possesses some local anaesthetic activity and this may

complicate the interpretation of the results obtained with high doses of this drug. For this reason, the effects on arrhythmias of another selective α_2-adrenoceptor antagonist idazoxan were also investigated, both when given alone and in combination with DMI.

Idazoxan, at 0.03 mg/kg, produced small but significant increases in blood pressure and heart rate and a significant reduction in the number of PVCs from 741 $\pm$ 93 in the control group to 321 $\pm$ 93. This dose, however, had no significant effect on the more serious arrhythmias, with the incidences of VT and VF remaining very similar to the control values at 75% and 25% respectively. There was also no effect on the incidence of mortality. At the 0.3 mg/kg dose, which produced significant increases in blood pressure and heart rate, idazoxan did not affect the number of PVCs and the incidence of VT but produced a significant increase in the incidence of VF from 29% in the control group to 56%. There was also a small increase in mortality but this was not statistically significant (Figure 29).

Similar to the results obtained with 0.1 mg/kg yohimbine, concomitant administration of 0.1 mg/kg DMI with 0.3 mg/kg idazoxan produced very large increases in blood pressure and heart rate and abolished the arrhythmogenic effect of this dose of idazoxan. There was, in fact, a significant decrease in the number of PVCs to 361 $\pm$ 96. The incidences of VT, VF and mortality in this group were not significantly different from controls at 71%, 36% and 0% respectively (Figure 29).

Idazoxan did not affect the onset of VT either alone or in combination with DMI. The duration of VT was reduced from 55 $\pm$ 8 s in the control group to 20 $\pm$ 6 s by both idazoxan alone at 0.03 mg/kg

and the combination of 0.3 mg/kg idazoxan with 0.1 mg/kg DMI but these reductions were not statistically significant (0.1>P>0.05). The reductions in the duration of VT were reflected by significant reductions in the number of PVCs in these groups, as shown in figure 29. The onset and duration of ventricular fibrillation were not significantly altered by idazoxan or the combination of idazoxan and DMI (Table 4).

Figure 30 shows the distribution of PVCs in the 20 min period following coronary occlusion in the control group and groups treated with idazoxan, either alone or in combination with DMI. The distribution of PVCs was not altered by any of the treatments, with the majority of PVCs occurring between 4 and 15 min after coronary occlusion and with peak ectopic activity at around 8 min, although the number of PVCs was suppressed by two of the treatments, as mentioned above.

4.8. Arrhythmias in vitamin E deficient rats

The total number of PVCs in the vitamin E deficient group was significantly lower than the control value of 741 $\pm$ 93, at 289 $\pm$ 81. The incidences of VT, VF and mortality, however, were not significantly altered at 67%, 20% and 7% respectively (Figure 31). The reduction in the number of PVCs resulted from a significant reduction in the duration of VT in the vitamin E deficient group, which measured 19 $\pm$ 8 s compared to 55 $\pm$ 8 s in the control group (Table 5). The onset of VT and the onset and duration of VF were not significantly different from control. The distribution of PVCs following coronary occlusion was similar in control and vitamin E deficient groups, although the total number of PVCs was suppressed in

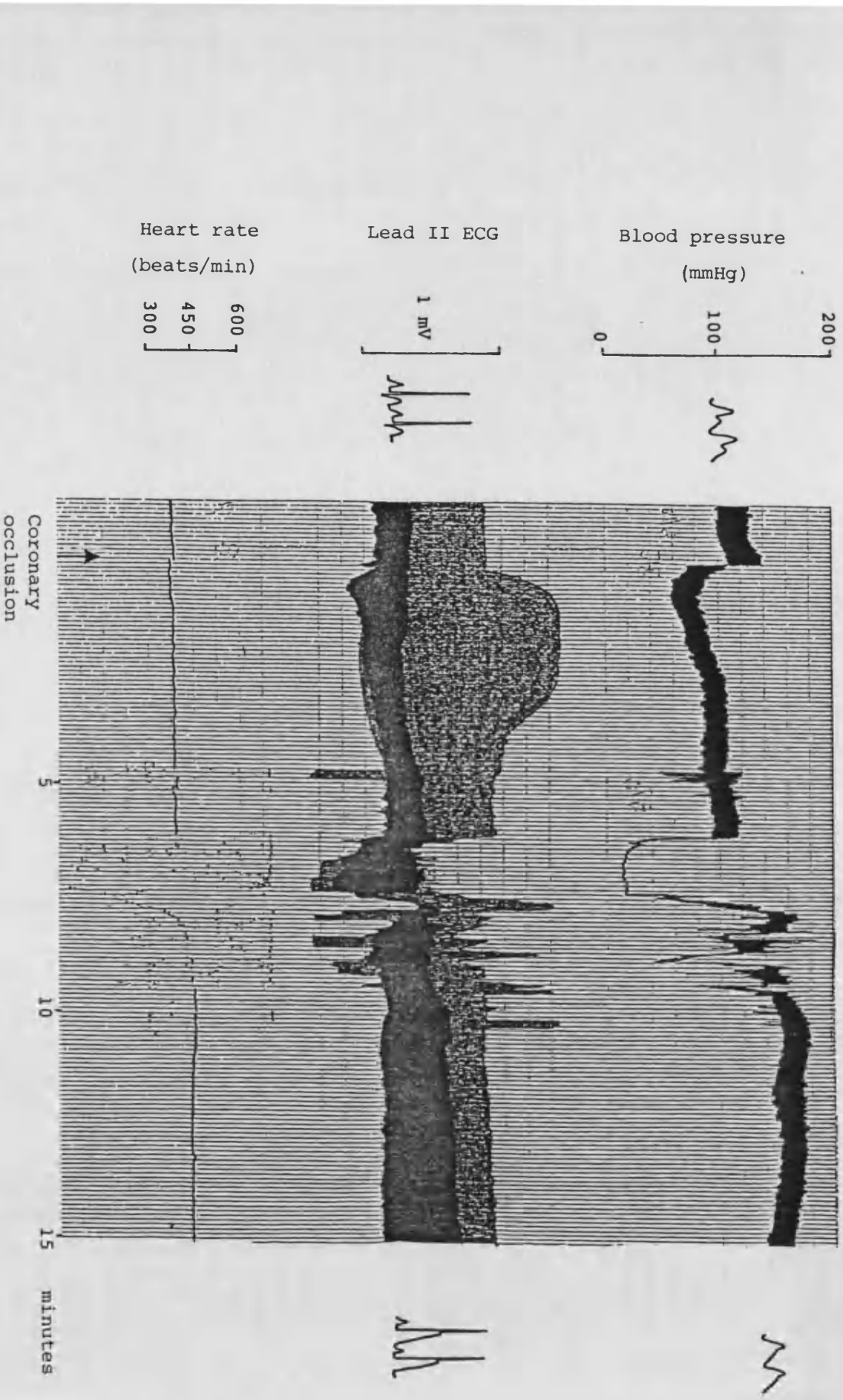
the latter group (Figure 32).

4.9. Effects of blood pressure and heart rate on arrhythmias

The mean arterial pressure (MAP) and heart rate (HR) values at the time of coronary occlusion were plotted against the arrhythmia score (calculated from the arbitrary scale described in section 2.1.(2)) for individual experiments in each group. No significant correlation was observed between either of these parameters and the severity of arrhythmias.

The scatter diagrams in Figure 33 show the lack of correlation between MAP or HR at the time of occlusion and the severity of subsequent arrhythmias, in the control group. Similar results were obtained in the treatment groups.

Figure 17 Representative recording from a control experiment showing the effects of coronary occlusion on blood pressure, lead II electrocardiogram (ECG) and heart rate.


Heart rate
(beats/min)
300
450
600
Lead II ECG
1 mV
Blood pressure
(mmHg)
0
100
200
Coronary
occlusion
5
10
15
minutes

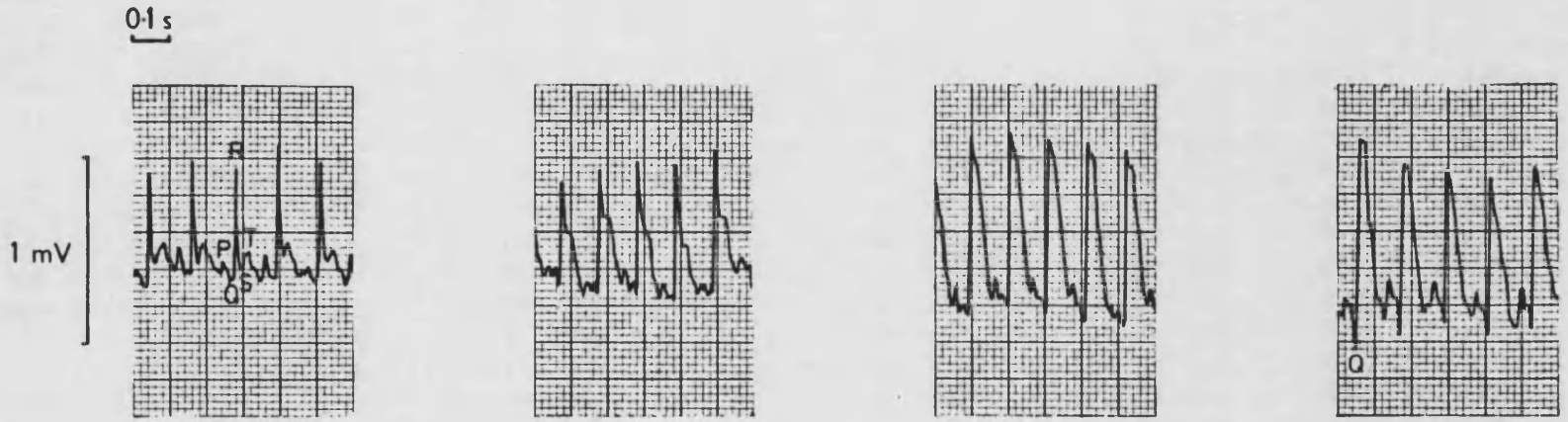
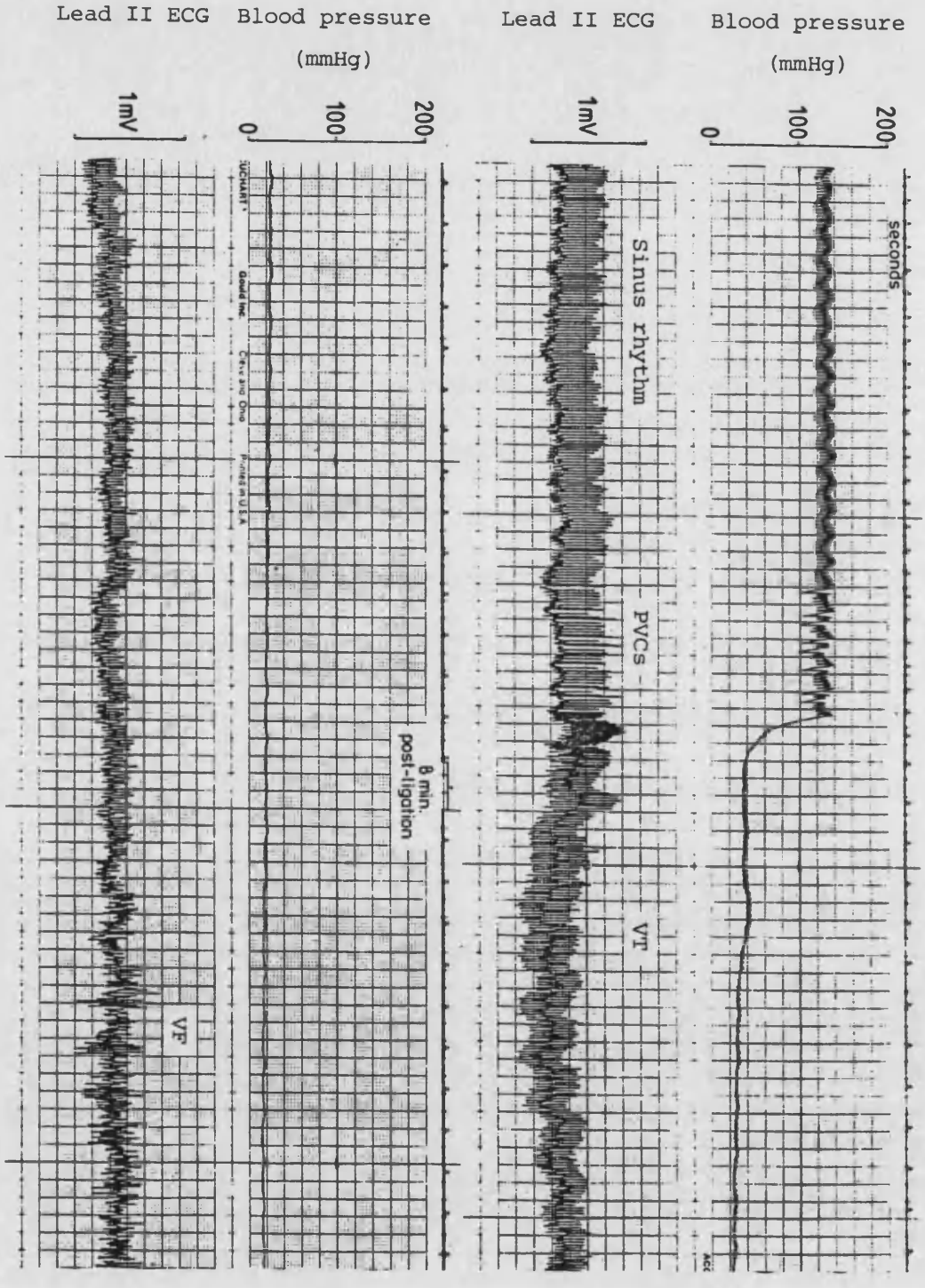


Figure 18 High speed lead II electrocardiogram (ECG) recordings from a control experiment before and at intervals after coronary occlusion.

Figure 19 Representative high speed blood pressure and lead II electrocardiogram (ECG) recordings from a control experiment illustrating the various types of arrhythmias observed in this model. PVCs=premature ventricular contractions, VT=ventricular tachycardia, VF=ventricular fibrillation.


Lead II ECG
Blood pressure
(mmHg)
1mV
0
100
200
seconds
Sinus rhythm
PVCs
VT
VF
8 min.
post-ligation

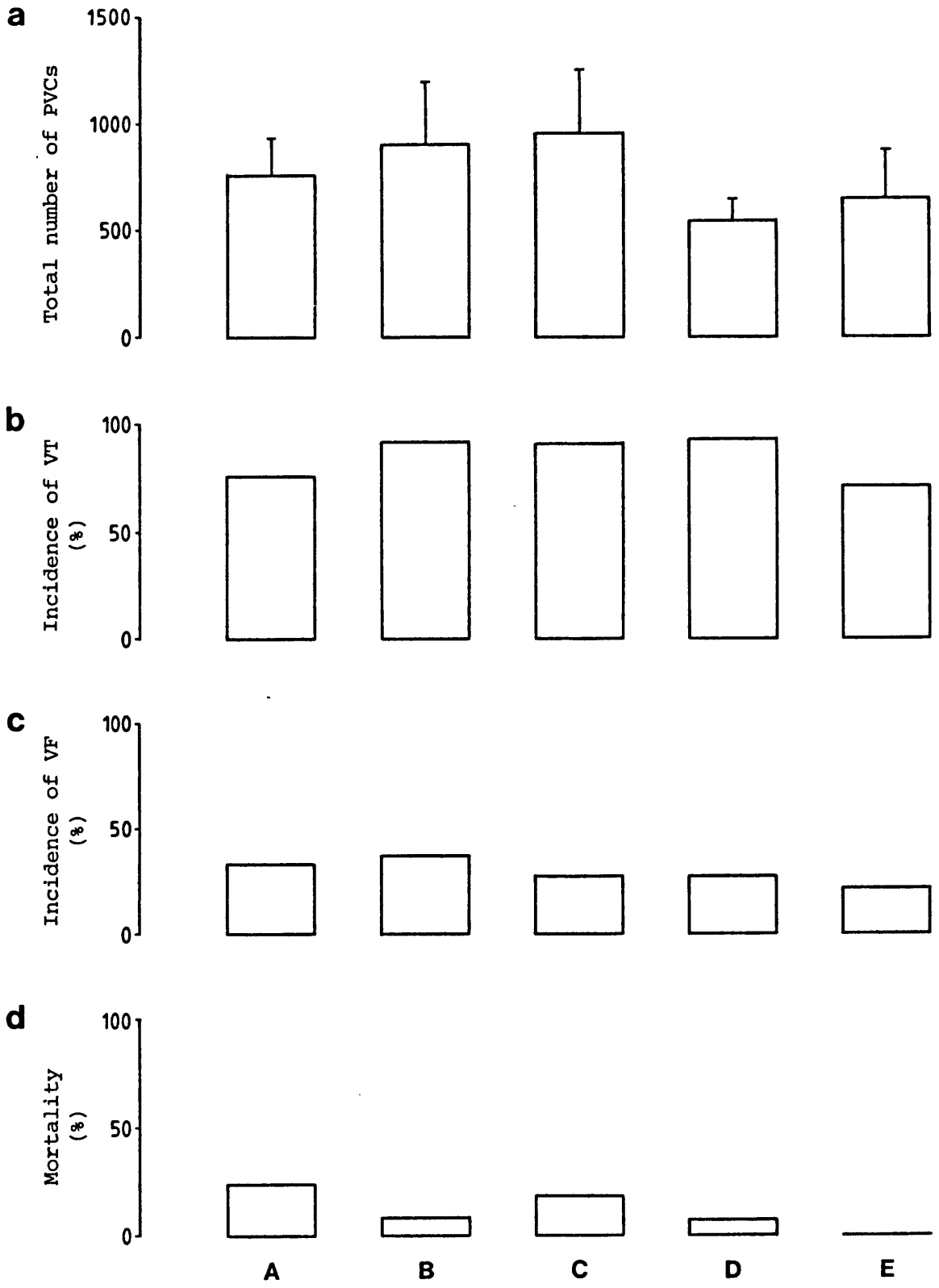


Figure 20 (a) The total number of premature ventricular contractions (PVCs) and the incidences of (b) ventricular tachycardia (VT), (c) ventricular fibrillation (VF), and (d) mortality in separate control groups (see section 4.2. for details).

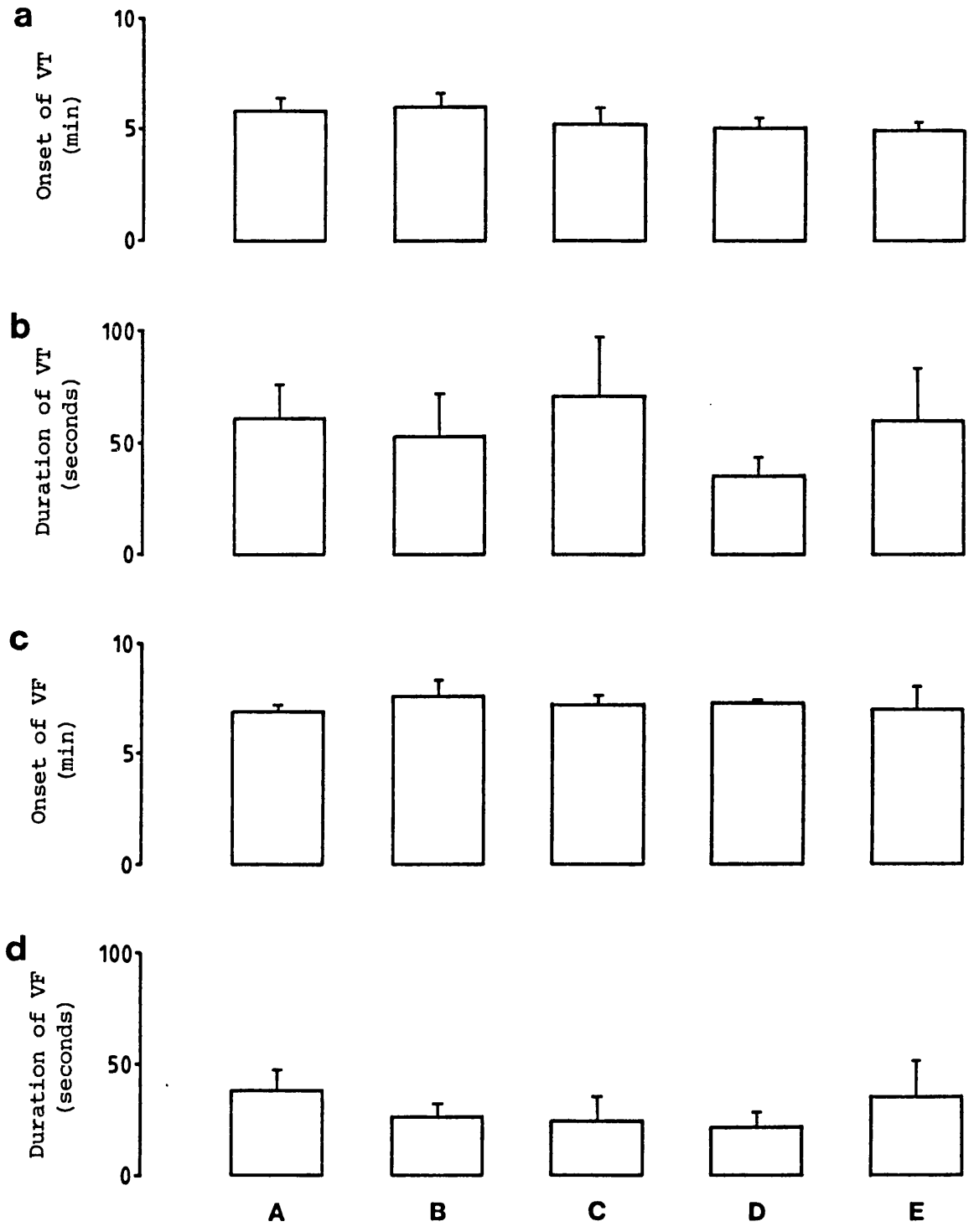
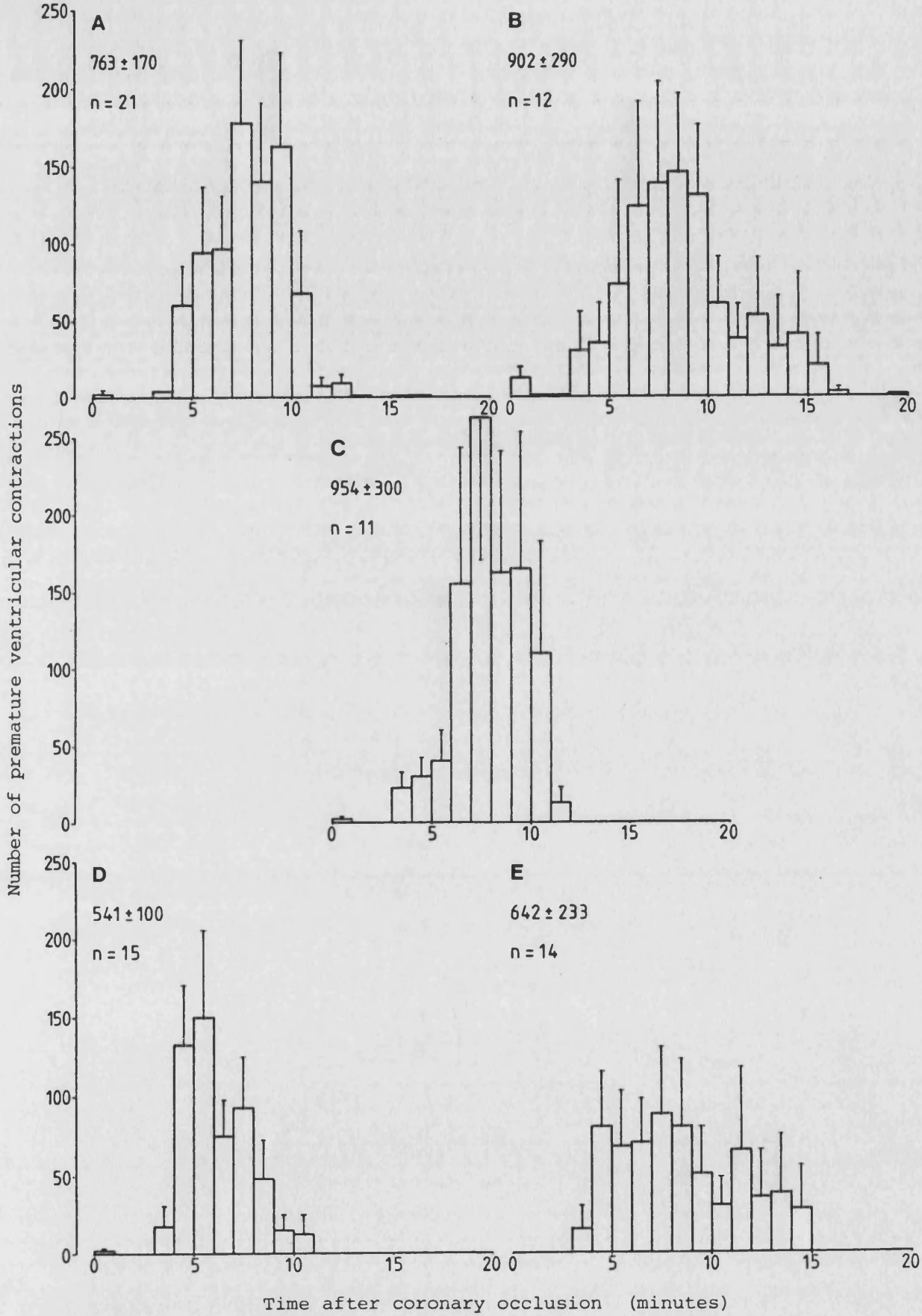


Figure 21 The onset and duration of ventricular tachycardia (VT,a and b) and ventricular fibrillation (VF,c and d) following coronary occlusion in separate control groups (see section 4.2. for details).

Figure 22 The distribution of premature ventricular contractions following coronary occlusion in separate control groups (see section 4.2. for details). The total number of premature ventricular contractions during the occlusion period is also indicated.


A
763 ± 170
n = 21
B
902 ± 290
n = 12
C
954 ± 300
n = 11
D
541 ± 100
n = 15
E
642 ± 233
n = 14
Number of premature ventricular contractions
Time after coronary occlusion (minutes)
0
50
100
150
200
250
5
10
15
20

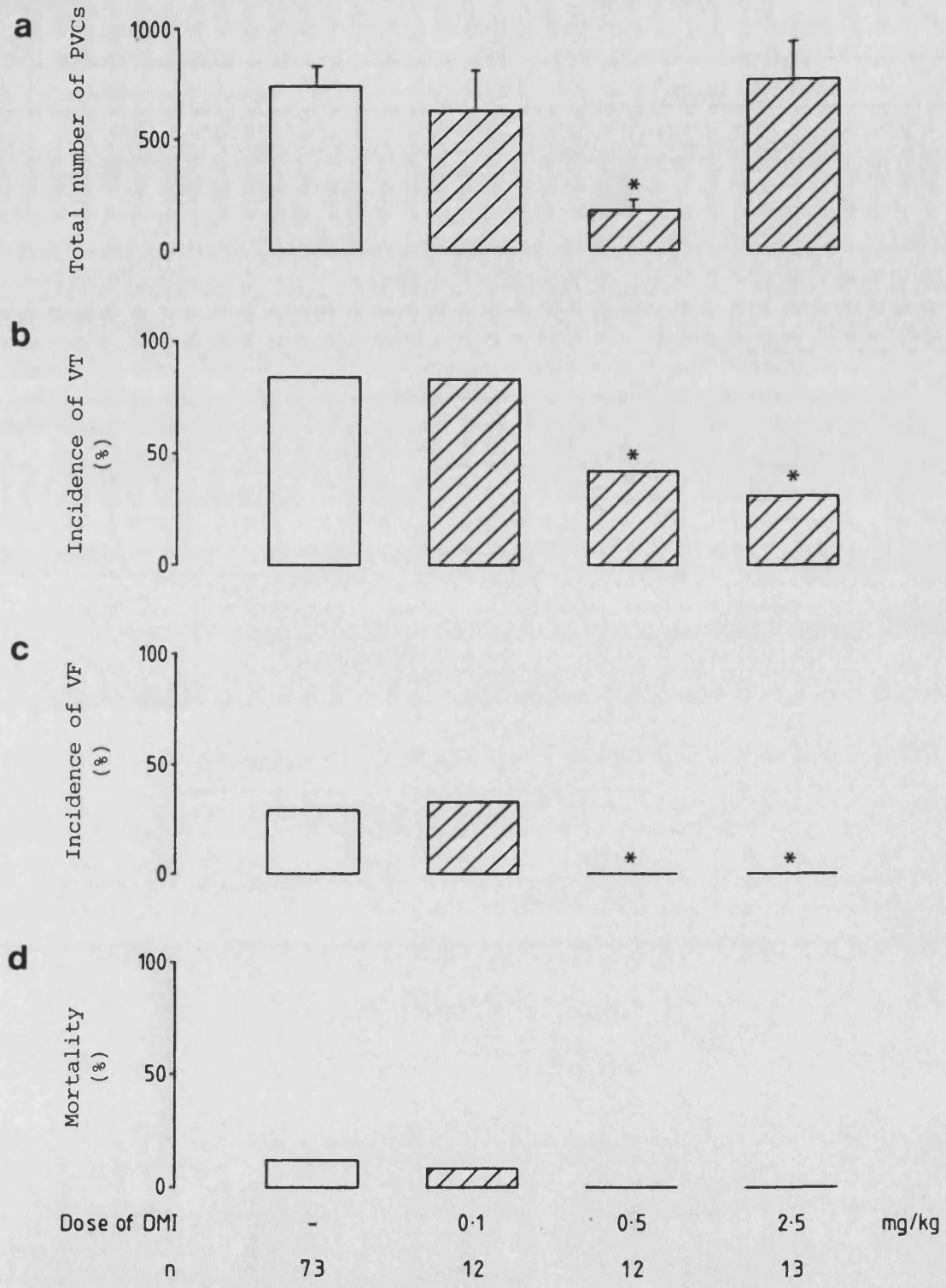


Figure 23 Effects of desipramine (DMI,hatched columns) on (a) the total number of premature ventricular contractions (PVCs) and the incidences of (b) ventricular tachycardia (VT), (c) ventricular fibrillation (VF), and (d) mortality. Open columns represent the control group and * denotes significant difference from control ($P<0.05$).

Drug	Dose (mg/kg)	n	Ventricular tachycardia			Ventricular fibrillation		
			n	Onset(min)	Duration(s)	n	Onset(min)	Duration(s)
Saline	-	73	61	5.4(0.3)	55(8)	21	7.2(0.2)	30(4)
DMI	0.1	12	10	4.9(0.6)	32(8)	4	5.6(0.6)	40(13)
DMI	0.5	12	5	6.7(1.3)	11(5)*	0	-	-
DMI	2.5	13	4	4.3(0.6)	59(31)	0	-	-

Table 1 Effects of desipramine (DMI) on the onset and duration of ventricular tachycardia and ventricular fibrillation following coronary occlusion. All values are means and values in brackets represent s.e.mean. * denotes significant difference from the control group treated with saline ($P<0.05$).

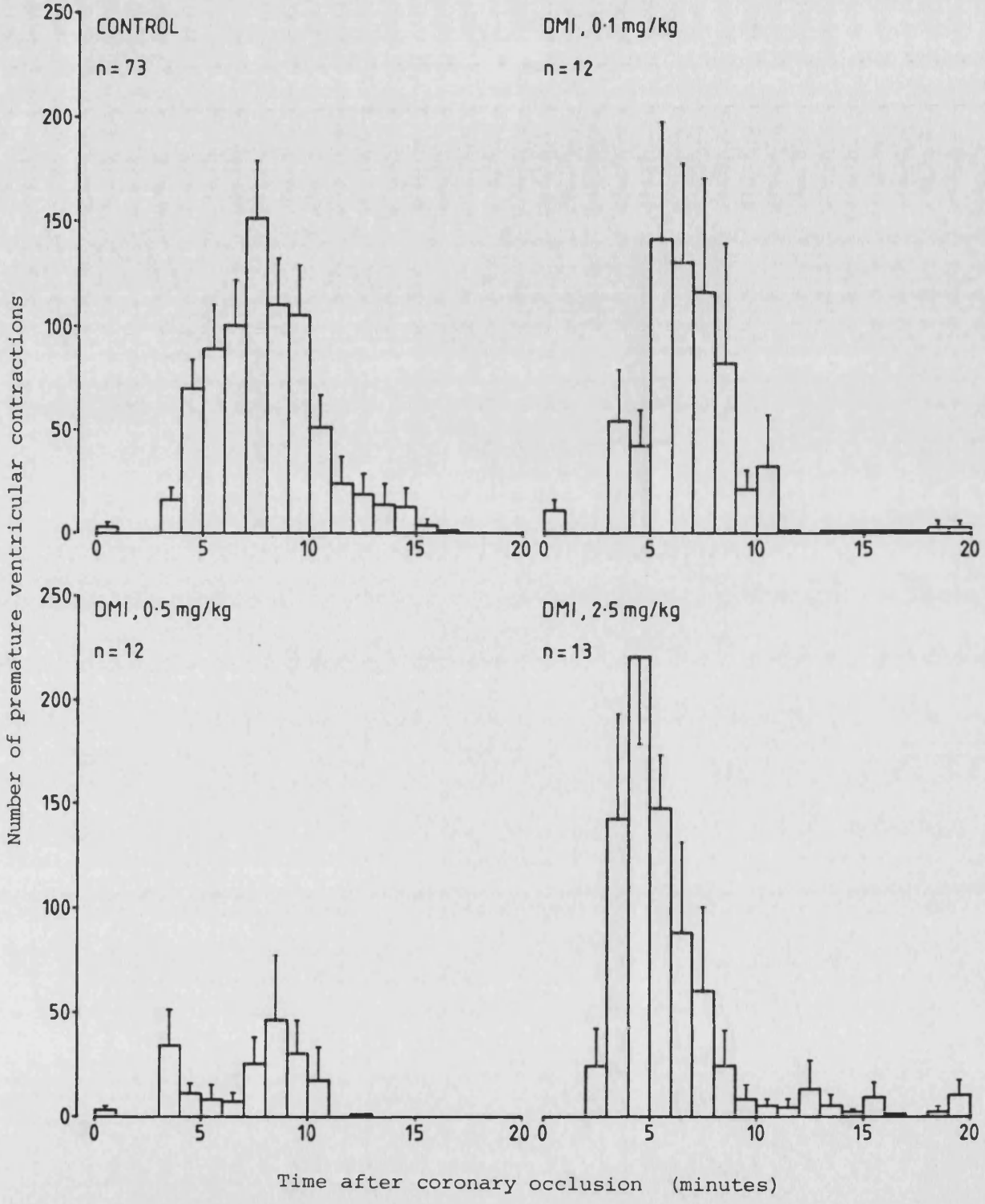


Figure 24 The distribution of premature ventricular contractions following coronary occlusion in the control group and groups receiving 0.1, 0.5 and 2.5 mg/kg doses of desipramine (DMI).

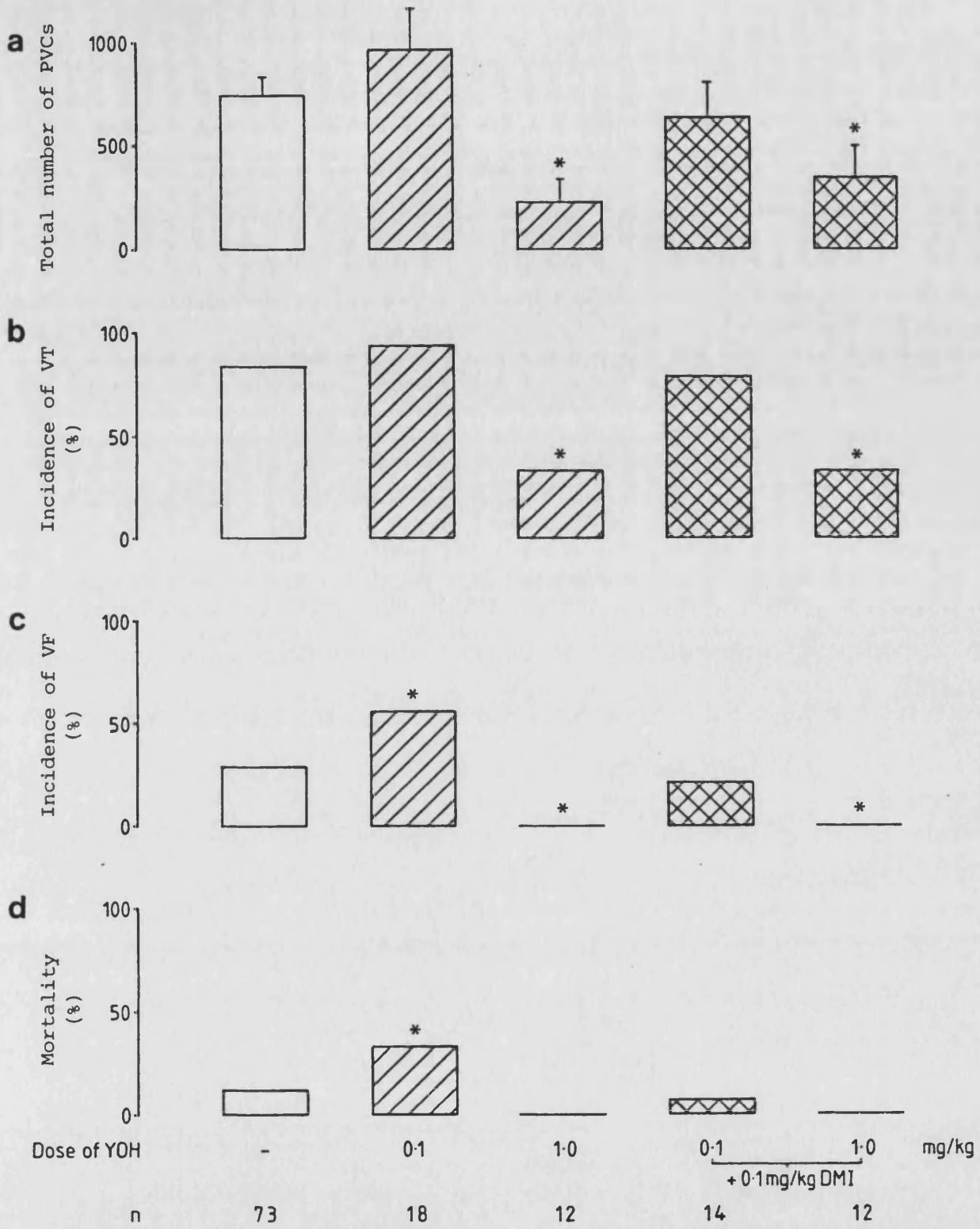
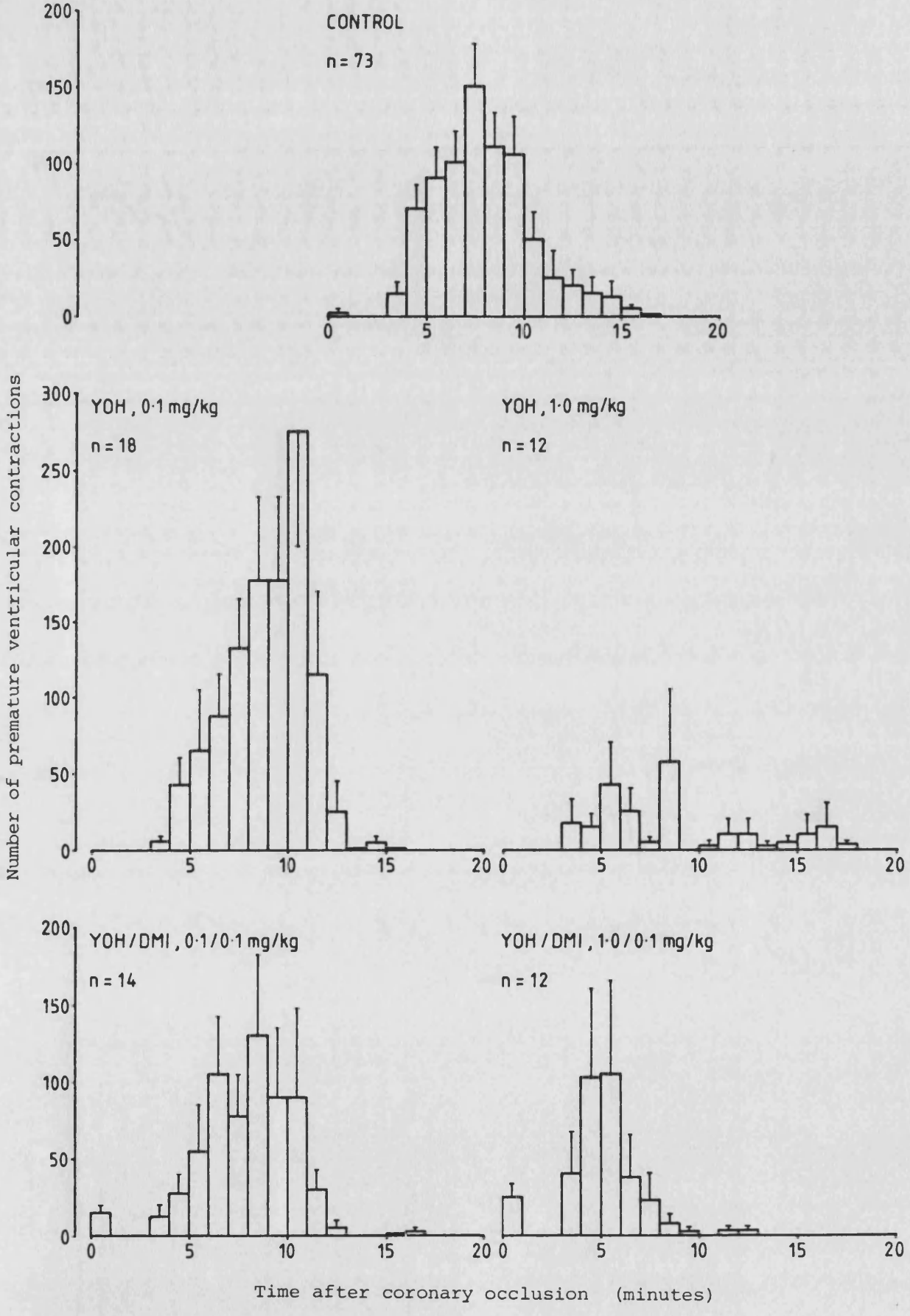


Figure 25 Effects of yohimbine, alone (YOH,hatched columns) and in combination with desipramine (YOH+DMI,cross-hatched columns), on (a) the total number of premature ventricular contractions (PVCs) and the incidences of (b) ventricular tachycardia (VT), (c) ventricular fibrillation (VF), and (d) mortality. Open columns represent the control group and * denotes significant difference from control ($P<0.05$).

Drug	Dose (mg/kg)	n	Ventricular tachycardia			Ventricular fibrillation		
			n	Onset(min)	Duration(s)	n	Onset(min)	Duration(s)
Saline	-	73	61	5.4(0.3)	55(8)	21	7.2(0.2)	30(4)
YOH	0.1	18	17	5.9(0.4)	67(18)	10	8.1(0.5)	41(12)
YOH	1.0	12	4	5.3(1.1)	36(11)	0	-	-
YOH/DMI	0.1/0.1	14	11	5.9(0.8)	45(18)	3	7.0(1.0)	50(6)
YOH/DMI	1.0/0.1	12	4	4.1(0.4)	58(20)	0	-	-

Table 2 Effects of yohimbine, alone (YOH) and in combination with desipramine (YOH/DMI), on the onset and duration of ventricular tachycardia and ventricular fibrillation following coronary occlusion. All values are means and values in brackets represent s.e.mean.

Figure 26 The distribution of premature ventricular contractions following coronary occlusion in the control group and groups receiving yohimbine, alone (YOH) or in combination with desipramine (YOH/DMI).


CONTROL
n = 73
YOH , 0·1 mg/kg
n = 18
YOH , 1·0 mg/kg
n = 12
YOH / DMI , 0·1 / 0·1 mg/kg
n = 14
YOH / DMI , 1·0 / 0·1 mg/kg
n = 12
Number of premature ventricular contractions
Time after coronary occlusion (minutes)

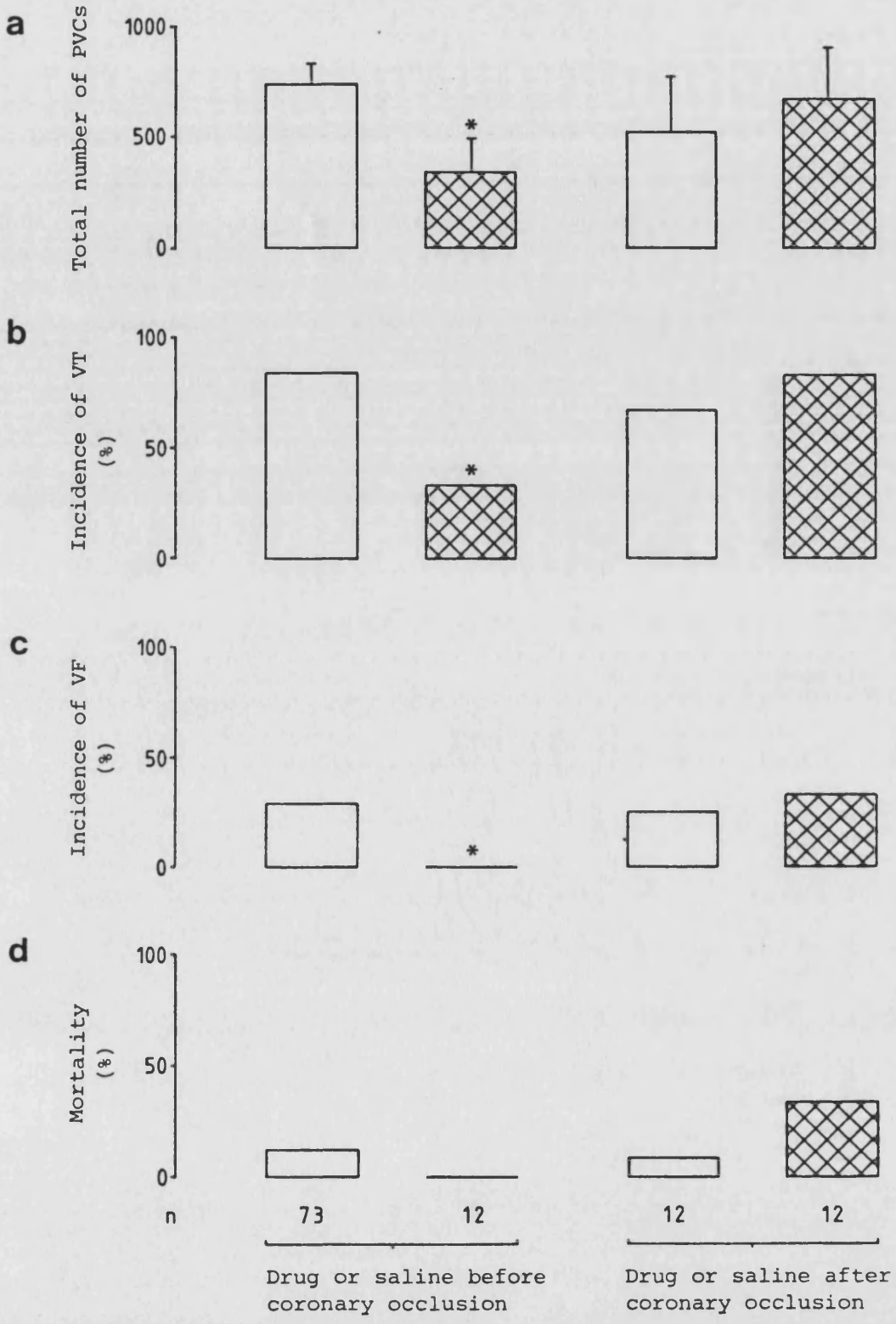


Figure 27 Effects of pre- and post-occlusion administration of a yohimbine/desipramine combination (1.0/0.1 mg/kg, cross-hatched columns) on (a) the total number of premature ventricular contractions (PVCs) and the incidences of (b) ventricular tachycardia (VT), (c) ventricular fibrillation (VF), and (d) mortality. Open columns represent control groups and * denotes significant difference from control ($P<0.05$).

Drug	Dose (mg/kg)	n	Ventricular tachycardia			Ventricular fibrillation		
			n	Onset (min)	Duration (s)	n	Onset (min)	Duration (s)
Saline	-	12	8	5.1 (0.4)	59 (35)	3	7.2 (0.3)	23 (16)
YOH/DMI	1.0/0.1	12	10	5.3 (0.3)	69 (23)	4	5.0 (0.4) *	15 (5)

Table 3 Effects of post-occlusion administration of a yohimbine/desipramine combination (YOH/DMI, 1.0/0.1 mg/kg) on the onset and duration of ventricular tachycardia and ventricular fibrillation. All values are means and values in brackets represent s.e.mean. * denotes significant difference from the control group receiving saline 2 min after coronary occlusion ($P<0.05$).

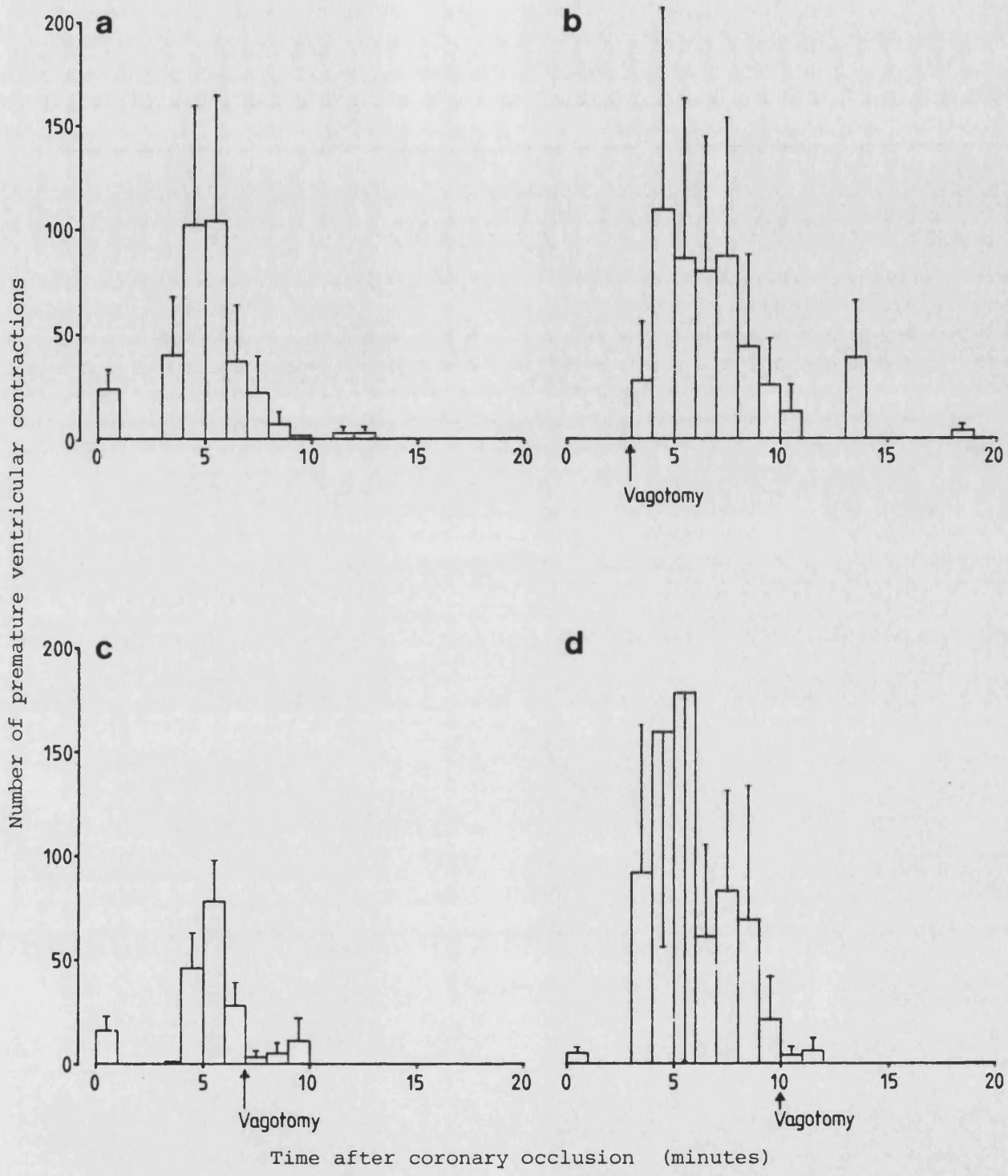


Figure 28 Effect of bilateral vagotomy on the occurence of premature ventricular contractions in rats treated with a yohimbine/desipramine combination (YOH/DMI,1.0/0.1 mg/kg) 5 min before coronary occlusion. (a) no vagotomy (n=12), (b) vagotomy at 3 min post-occlusion (n=4), (c) vagotomy at 7 min post-occlusion (n=4), and (d) vagotomy at 10 min post-occlusion (n=3).

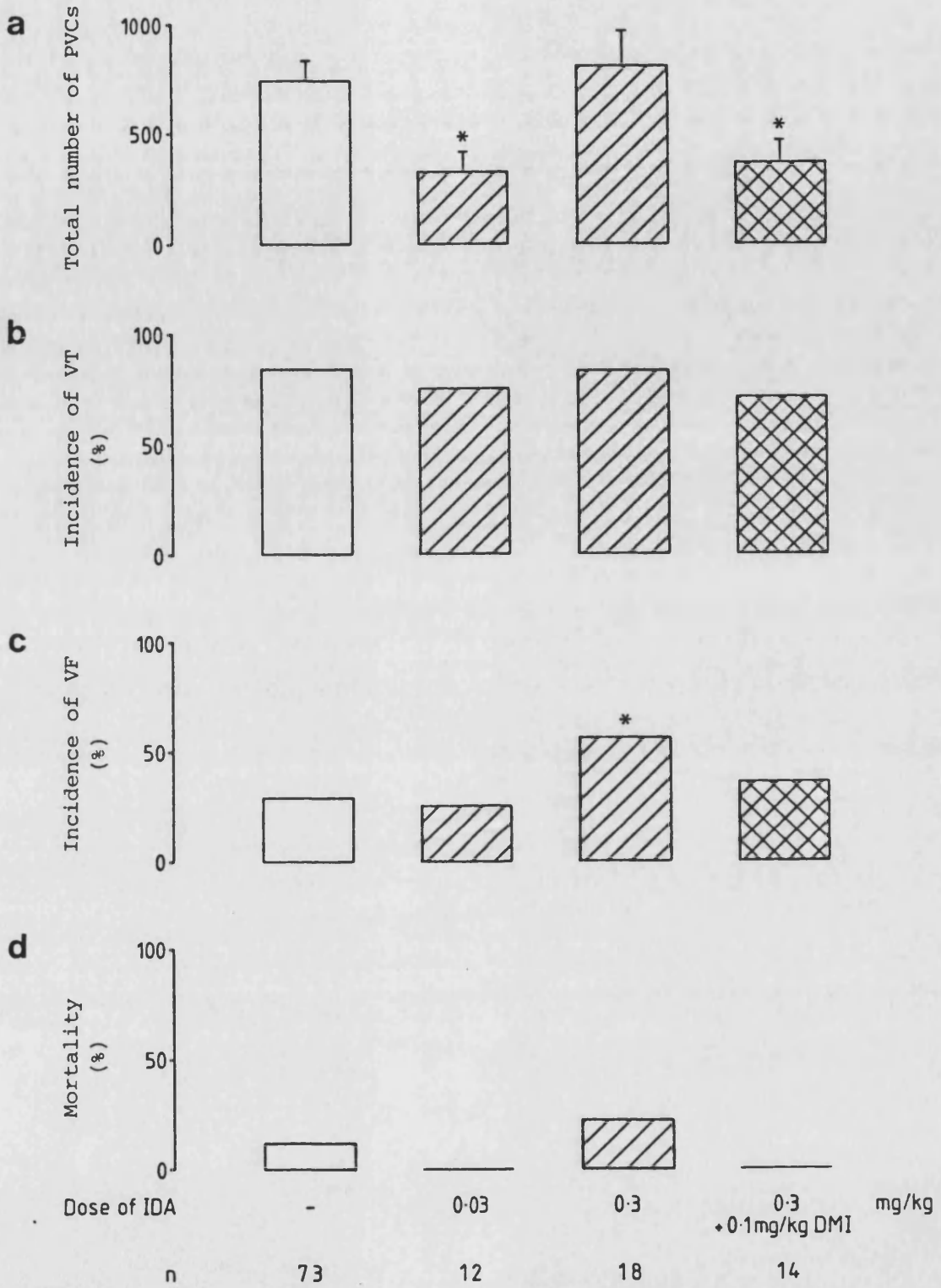


Figure 29 Effects of idazoxan, alone (IDA,hatched columns) and in combination with desipramine (IDA+DMI,cross-hatched columns), on (a) the total number of premature ventricular contractions (PVCs) and the incidences of (b) ventricular tachycardia (VT), (c) ventricular fibrillation (VF), and (d) mortality. Open columns represent the control group and * denotes significant difference from control ($P<0.05$).

Drug	Dose (mg/kg)	n	Ventricular tachycardia			Ventricular fibrillation		
			n	Onset(min)	Duration(s)	n	Onset(min)	Duration(s)
Saline	-	73	61	5.4(0.3)	55(8)	21	7.2(0.2)	30(4)
IDA	0.03	12	9	5.1(0.5)	20(6)	3	7.5(0.9)	36(26)
IDA	0.3	18	15	5.2(0.4)	62(17)	10	6.8(0.5)	32(13)
IDA/DMI	0.3/0.1	14	10	4.8(0.3)	20(6)	5	7.5(0.9)	35(17)

Table 4 Effects of idazoxan, alone (IDA) and in combination with desipramine (IDA/DMI), on the onset and duration of ventricular tachycardia and ventricular fibrillation following coronary occlusion. All values are means and values in brackets represent s.e.mean. * denotes significant difference from the control group treated with saline ($P<0.05$).

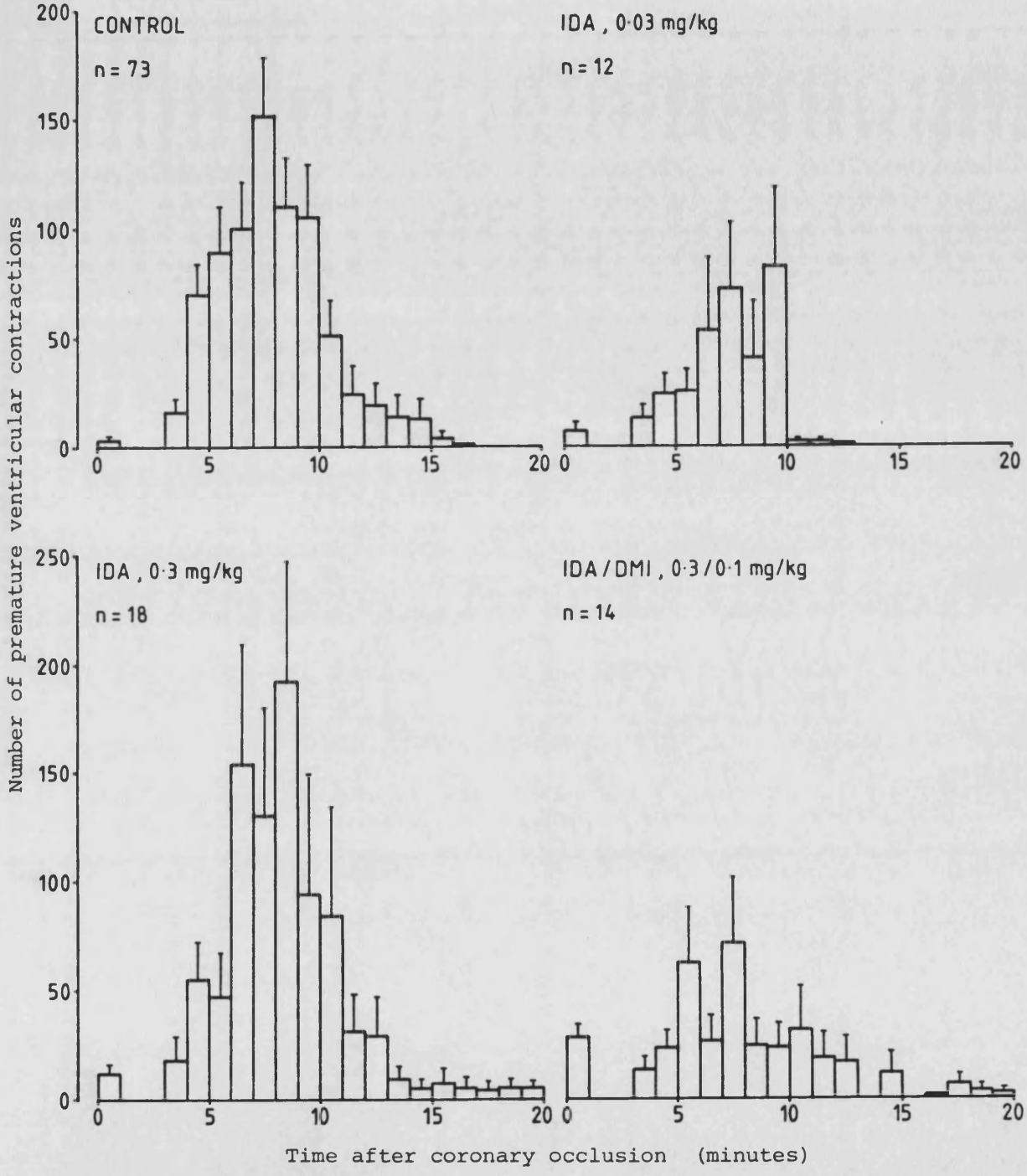


Figure 30 The distribution of premature ventricular contractions following coronary occlusion in the control group and groups receiving idazoxan, alone (IDA) and in combination with desipramine (IDA/DMI).

Figure 31 (a) The total number of premature ventricular contractions (PVCs) and the incidences of (b) ventricular tachycardia (VT), (c) ventricular fibrillation (VF), and (d) mortality in control (open columns) and vitamin E deficient (hatched columns) groups. * denotes significant difference from control ($P<0.05$).

Group	n	Ventricular tachycardia			Ventricular fibrillation		
		n	Onset(min)	Duration(s)	n	Onset(min)	Duration(s)
Control	73	61	5.4(0.3)	55(8)	21	7.2(0.2)	30(4)
Vitamin E deficient	15	10	4.5(0.4)	19(8)*	3	6.7(0.5)	43(23)

Table 5 The onset and duration of ventricular tachycardia and ventricular fibrillation following coronary occlusion in control and vitamin E deficient groups. * denotes significant difference from control ($P<0.05$).

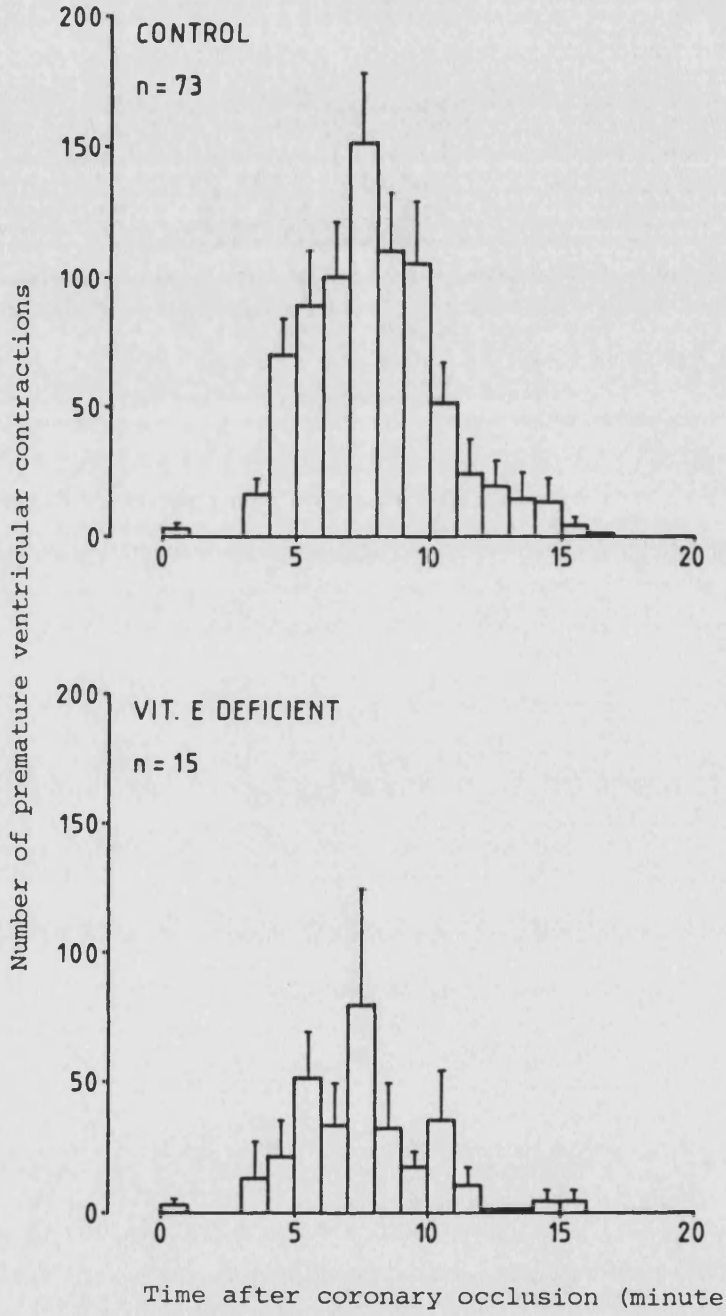


Figure 32 The distribution of premature ventricular contractions following coronary occlusion in control and vitamin E deficient groups.

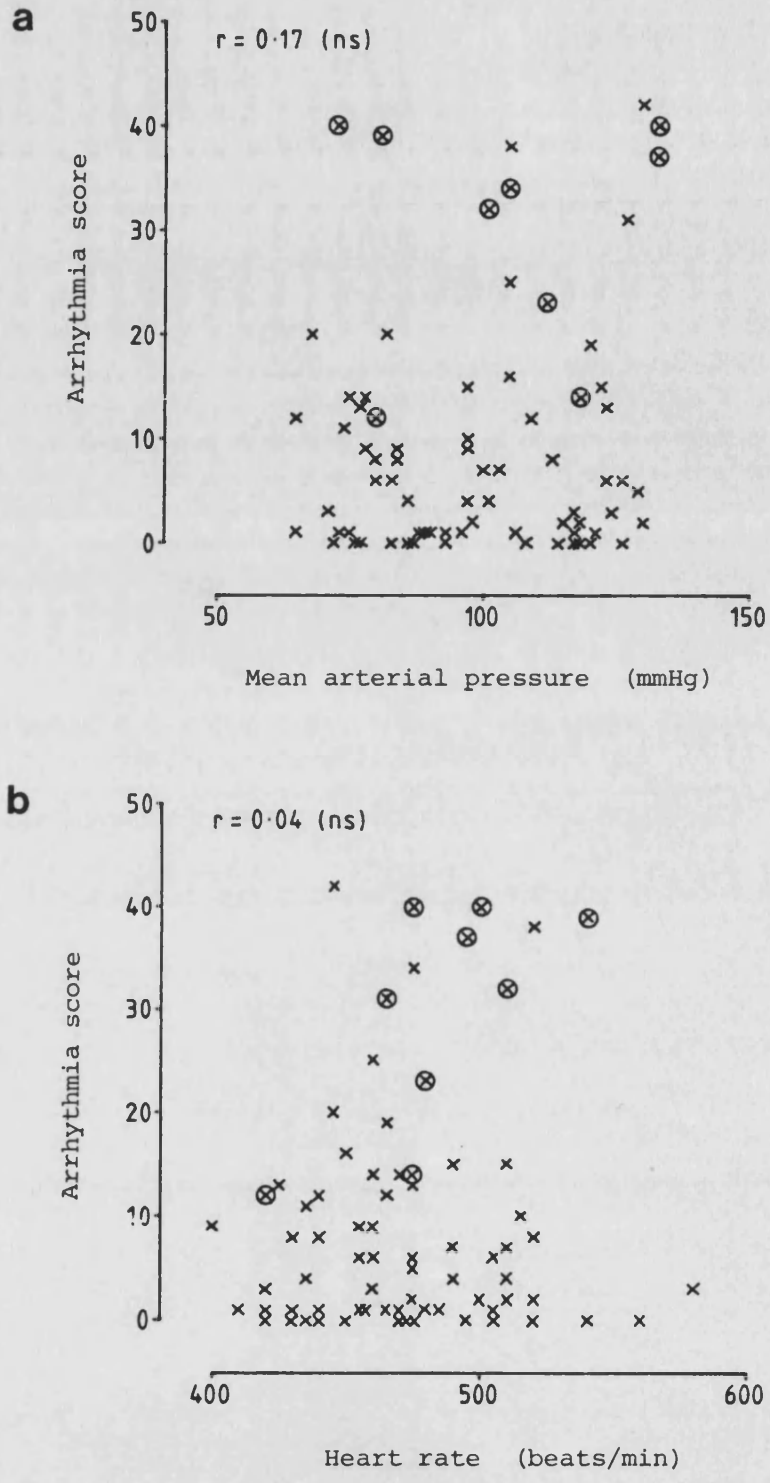


Figure 33 Scatter diagrams showing the lack of correlation between (a) mean arterial pressure, and (b) heart rate and the arrhythmia score in control experiments (n=73). Encircled symbols indicate the animals which died.

Chapter 5

EFFECTS ON PLASMA CATECHOLAMINE CONCENTRATIONS

5.1. Effects of coronary occlusion on the plasma concentrations of noradrenaline and adrenaline

The concentrations of noradrenaline and adrenaline in plasma samples obtained 3 minutes after coronary occlusion (control) or sham occlusion did not differ significantly. Sampling was carried out at 3 min post-occlusion because it corresponds to the period immediately prior to the development of arrhythmias in control experiments.

The mean plasma noradrenaline concentration in the control group was 4.5 ± 0.7 pmol/ml compared to 3.4 ± 0.7 pmol/ml in the sham occlusion group. The plasma adrenaline concentration was slightly higher in the control group at 12.7 ± 3.2 pmol/ml than in the sham-occlusion group, which measured 9.0 ± 2.4 pmol/ml. This difference, however, was not statistically significant. These results show that 3 minutes of myocardial ischaemia does not produce a significant change in plasma catecholamine levels (Figure 34).

5.2. Effects of desipramine on the plasma concentrations of noradrenaline and adrenaline 3 minutes after coronary occlusion

Desipramine (DMI), at 0.1 mg/kg, tended to increase the plasma noradrenaline concentration. Plasma noradrenaline measured 9.4 ± 2.6 pmol/ml in the group receiving this dose of DMI compared to 4.5 ± 0.7 pmol/ml in the control group treated with saline, but the difference was not statistically significant. 0.1 mg/kg DMI had no significant effect on the plasma adrenaline concentration.

Increasing the dose of DMI to 0.5 mg/kg had no effect on the plasma concentration of noradrenaline but tended to reduce the adrenaline concentration, from 12.7 ± 3.2 pmol/ml in the control group to 7.5 ± 1.9 pmol/ml. At the highest dose of 2.5 mg/kg, DMI

again had no significant effect on the plasma noradrenaline concentration. The plasma concentration of adrenaline, however, was significantly reduced to 6.2 ± 1.7 pmol/ml compared to 12.7 ± 3.2 pmol/ml in the control group (Figure 35).

5.3. Effects of yohimbine, alone and in combination with desipramine, on the plasma concentrations of noradrenaline and adrenaline 3 minutes after coronary occlusion

Yohimbine, at the 0.1 mg/kg dose, had no significant effect on plasma noradrenaline and adrenaline concentrations which, at 4.3 ± 0.6 and 11.2 ± 3.2 pmol/ml respectively, were similar to the control values of 4.3 ± 0.6 pmol/ml for noradrenaline and 8.2 ± 1.7 pmol/ml for adrenaline. Combining this dose of yohimbine with 0.1 mg/kg DMI, however, produced a significant increase in the plasma noradrenaline concentration to 6.4 ± 0.7 pmol/ml without a significant effect on plasma adrenaline (Figure 36).

The 1.0 mg/kg dose of yohimbine again had no significant effect on plasma catecholamine levels with the concentrations of noradrenaline and adrenaline measuring 5.6 ± 1.8 and 13.6 ± 2.4 pmol/ml respectively, compared to control values of 4.5 ± 0.7 pmol/ml for noradrenaline and 12.7 ± 3.2 pmol/ml for adrenaline. The combination of 1.0 mg/kg yohimbine with 0.1 mg/kg DMI produced a significant increase in the plasma concentration of noradrenaline to 10.1 ± 1.7 pmol/ml without significantly altering plasma adrenaline (Figure 37). The increase in plasma noradrenaline concentration produced by this yohimbine/DMI combination was greater than that produced by the combination of DMI with the lower dose of yohimbine,

illustrated in Figure 36.

The experiments using the two doses of yohimbine were carried out at different times and separate controls groups, each comprising 6 animals, were established with each set of experiments. Although the plasma concentration of noradrenaline was similar in both control groups at 4.3 ± 0.6 and 4.5 ± 0.7 pmol/ml, the plasma concentrations of adrenaline slightly differed at 8.2 ± 1.7 and 12.7 ± 3.2 pmol/ml. This difference between the two control groups, however, was not statistically significant.

5.4. Effects of idazoxan, alone and in combination with desipramine, on the plasma concentrations of noradrenaline and adrenaline 3 minutes after coronary occlusion

Idazoxan significantly increased the plasma concentration of noradrenaline from 4.3 ± 0.6 pmol/ml in the control group to 8.4 ± 0.6 pmol/ml when administered alone at the 0.3 mg/kg dose. The concentration of adrenaline remained very similar to the control value at 8.2 ± 1.4 pmol/ml.

When 0.3 mg/kg idazoxan was administered in combination with 0.1 mg/kg DMI, it caused an even greater increase in the plasma noradrenaline concentration. The plasma noradrenaline concentration measured 11.6 ± 2.1 pmol/ml in this group and was almost three times the control level of 4.3 ± 0.6 pmol/ml. The concentration of adrenaline was not significantly affected by this combination of idazoxan and desipramine and measured 10.4 ± 3.4 pmol/ml compared to 8.2 ± 1.7 pmol/ml in the control group (Figure 38).

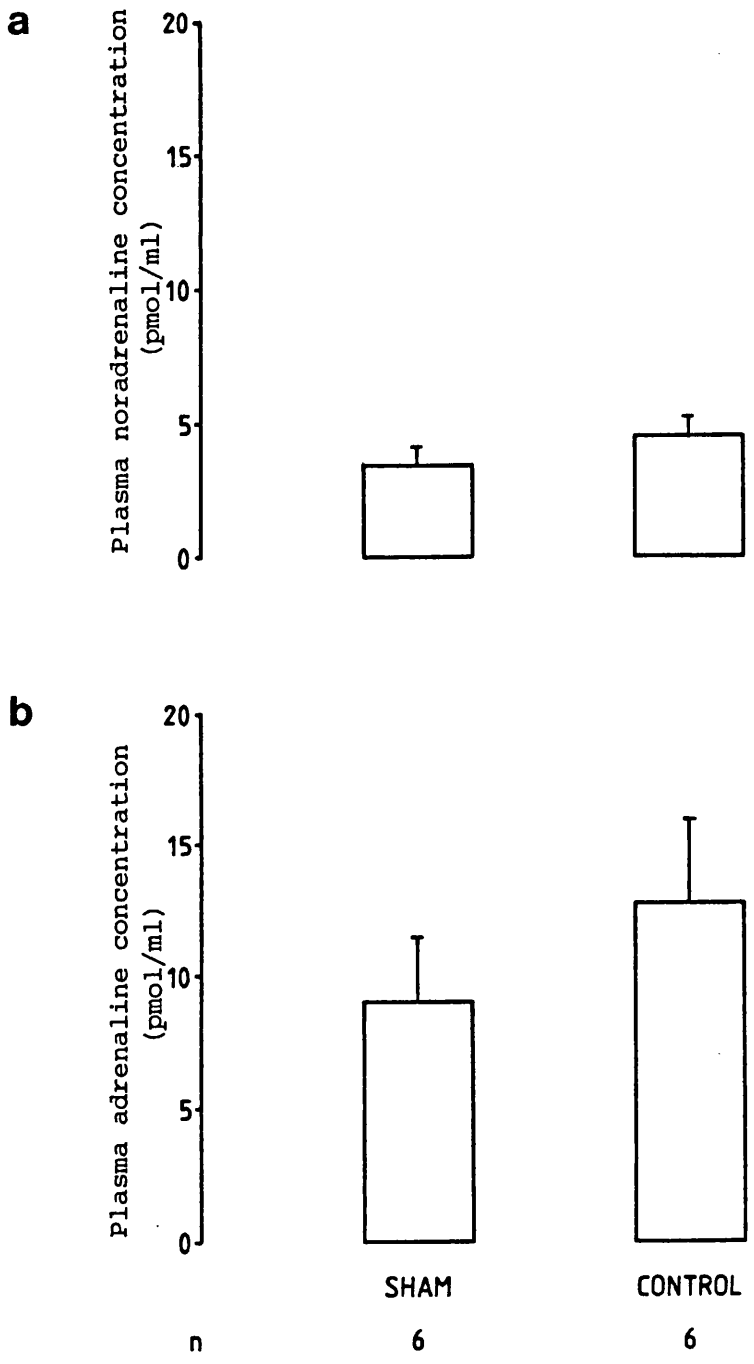


Figure 34 Plasma concentrations of (a) noradrenaline and (b) adrenaline 3 min after sham occlusion or coronary occlusion (control). All values are means and vertical lines represent s.e.mean.

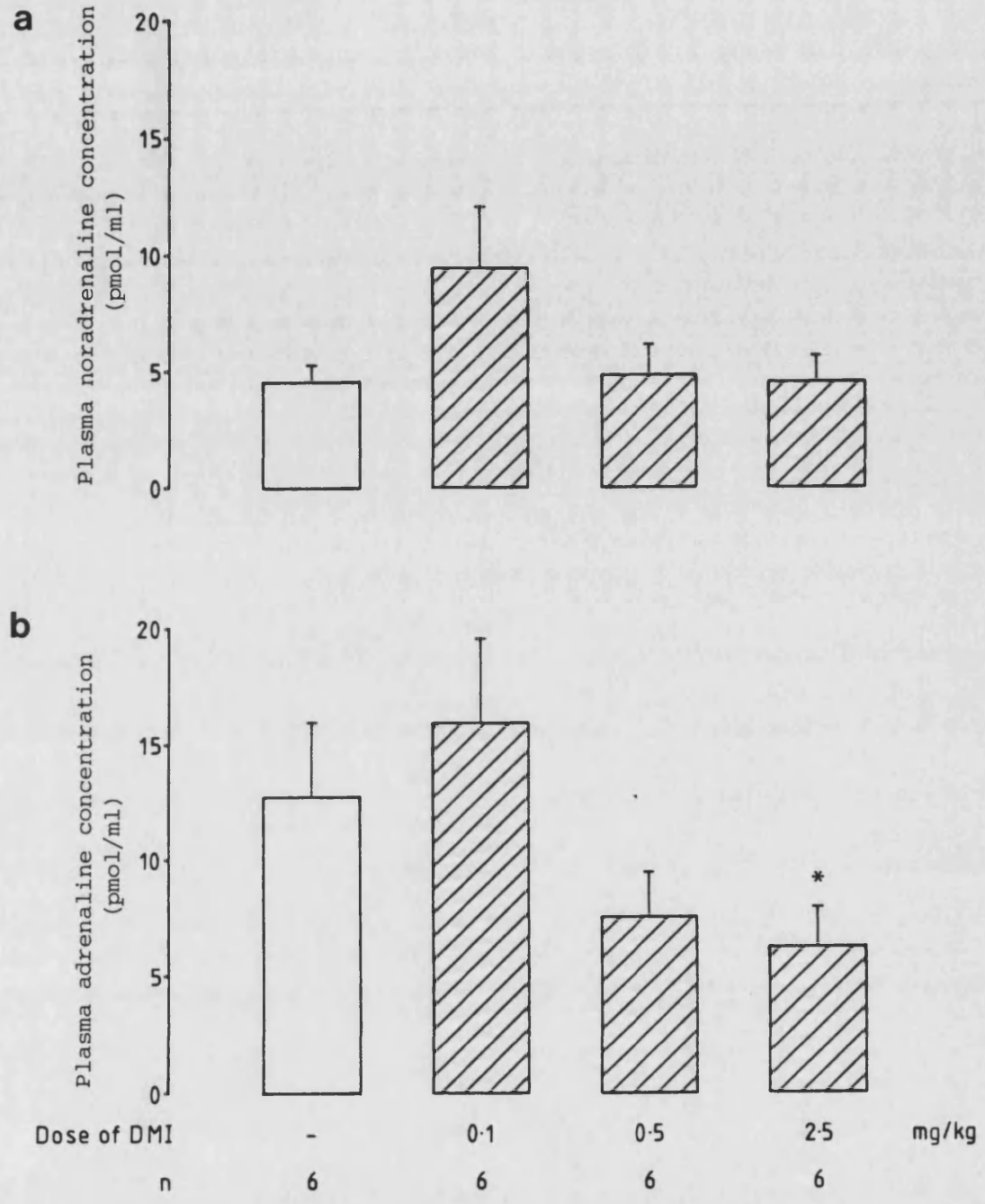


Figure 35 Effects of desipramine (DMI,hatched columns) on the plasma concentrations of (a) noradrenaline and (b) adrenaline 3 min after coronary occlusion. Open columns represent the control group and * denotes significant difference from control ($P<0.05$).

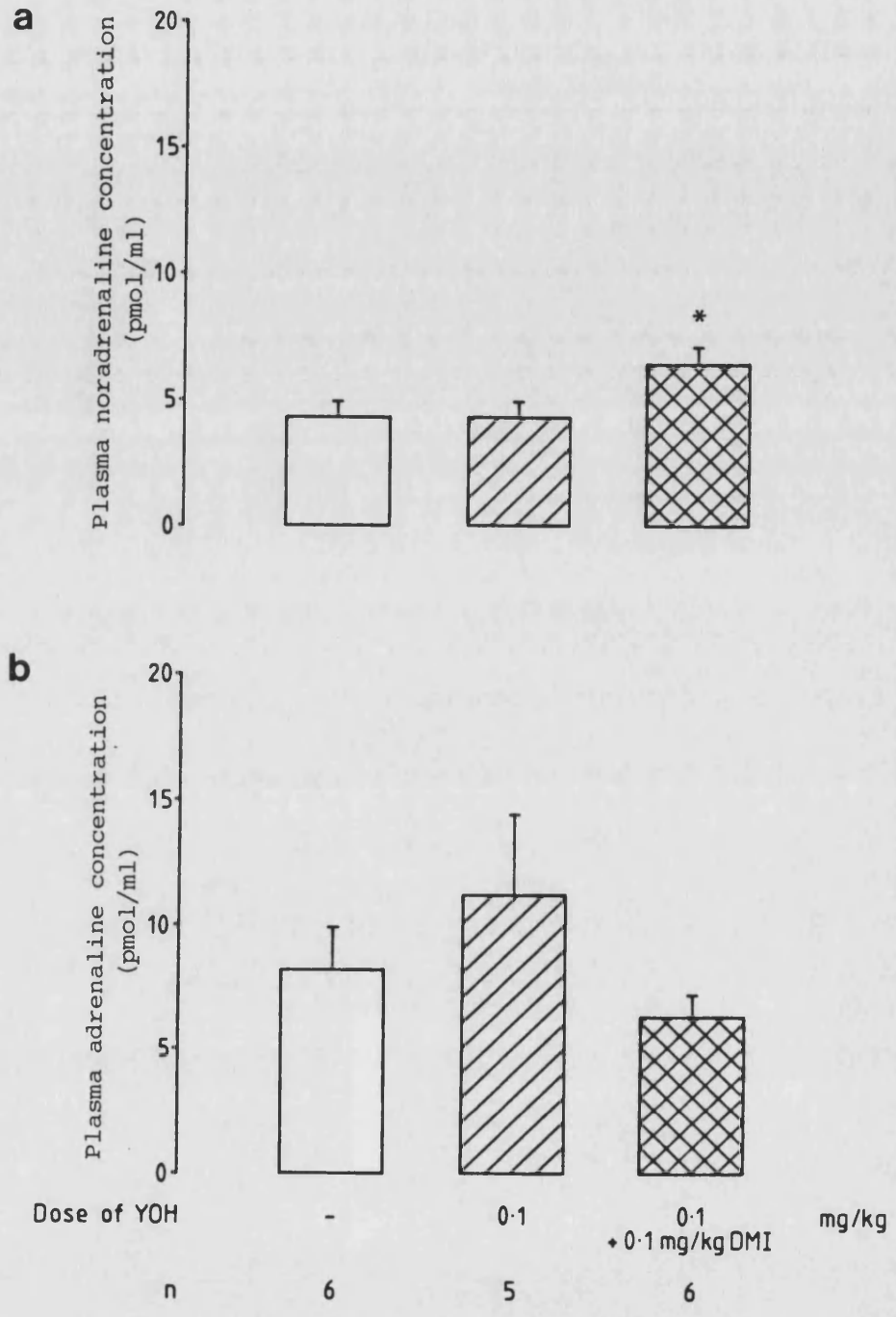


Figure 36 Effects of 0.1 mg/kg yohimbine, alone (YOH,hatched columns) and in combination with desipramine (YOH+DMI,cross-hatched columns), on the plasma concentrations of (a) noradrenaline and (b) adrenaline 3 min after coronary occlusion. Open columns represent the control group and * denotes significant difference from control ($P<0.05$).

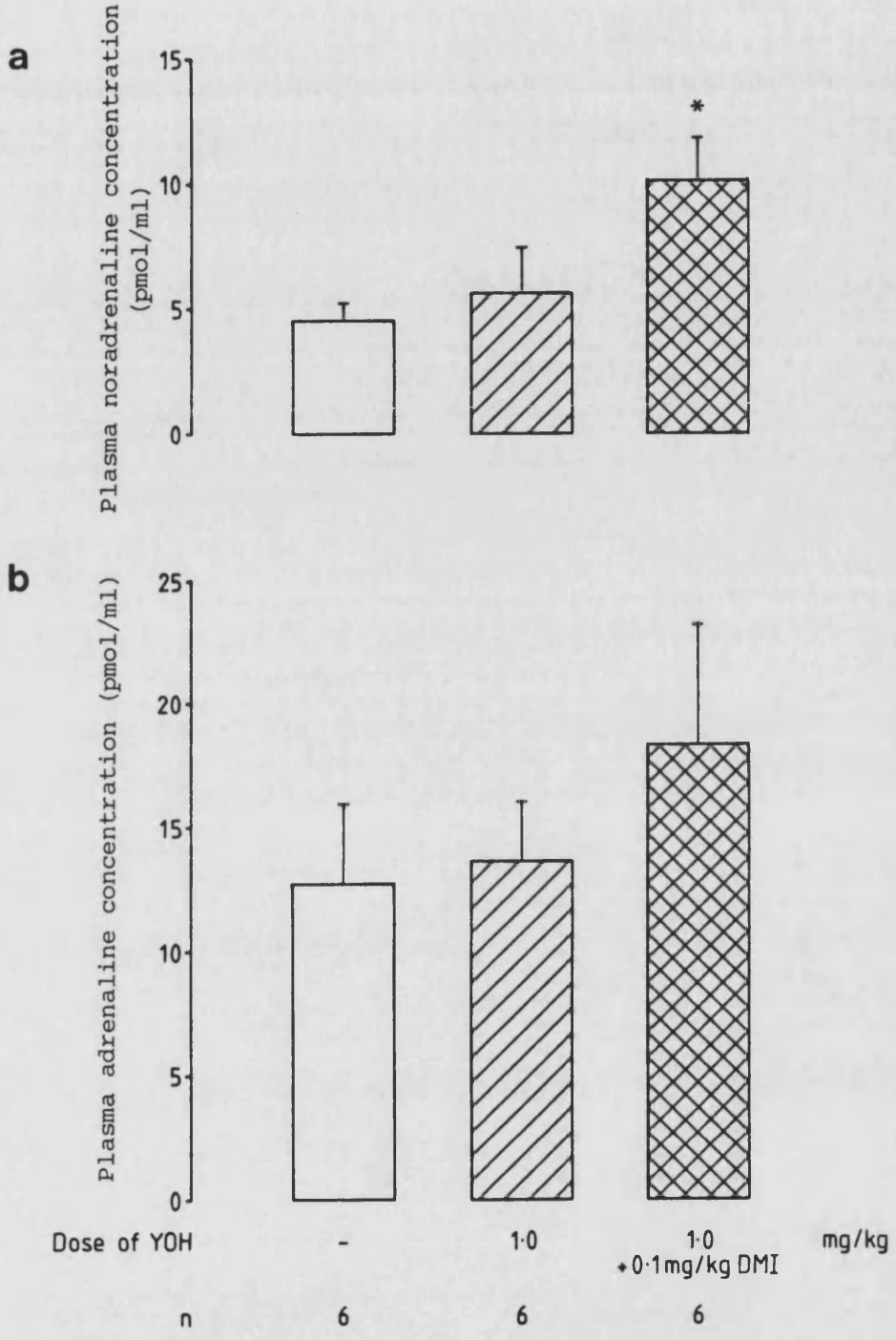


Figure 37 Effects of 1.0 mg/kg yohimbine, alone (YOH,hatched columns) and in combination with desipramine (YOH+DMI,cross-hatched columns), on the plasma concentrations of (a) noradrenaline and (b) adrenaline 3 min after coronary occlusion. Open columns represent the control group and * denotes significant difference from control ($P<0.05$).

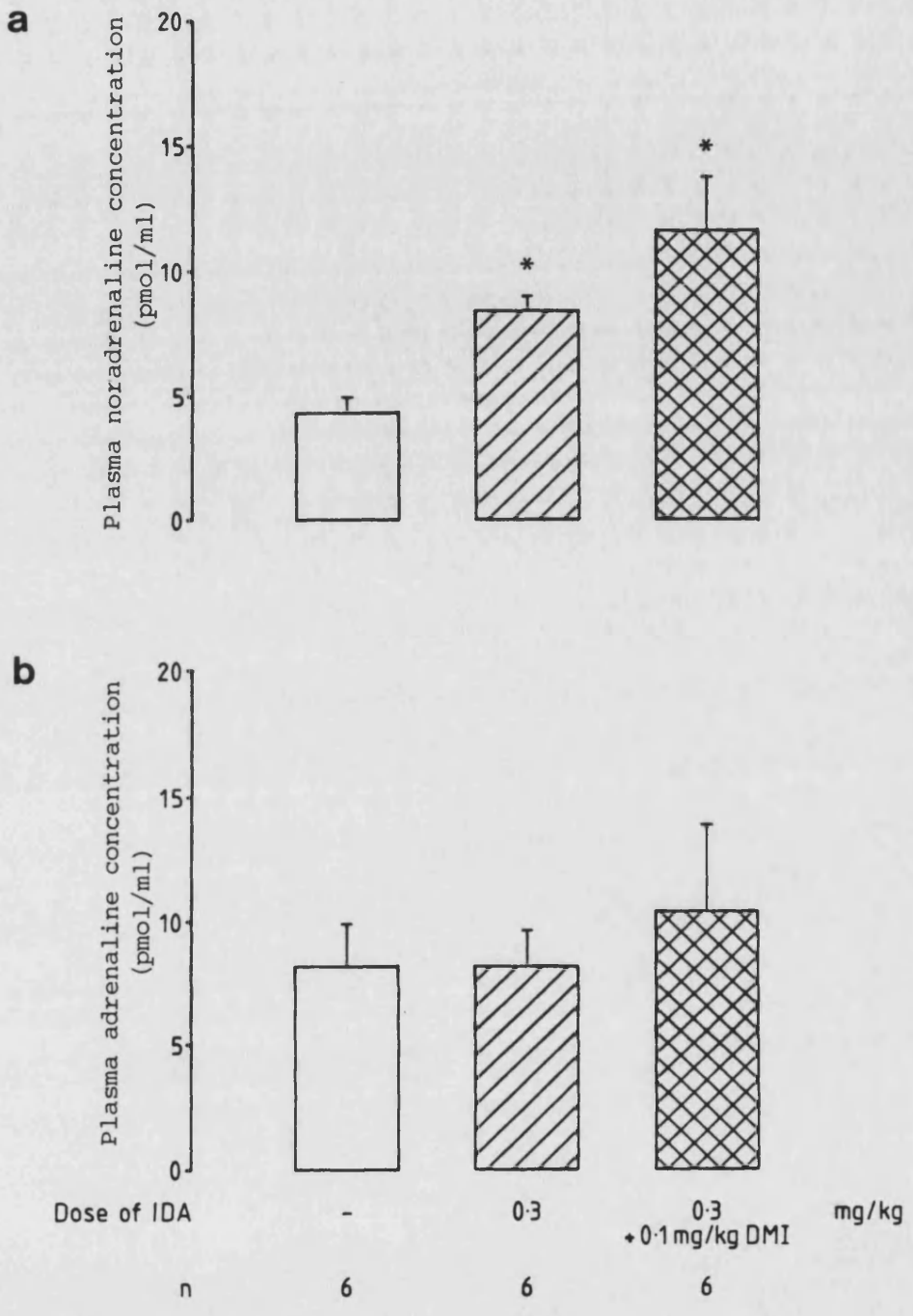


Figure 38 Effects of idazoxan, alone (IDA,hatched columns) and in combination with desipramine (IDA+DMI,cross-hatched columns), on the plasma concentrations of (a) noradrenaline and (b) adrenaline 3 min after coronary occlusion. Open columns represent the control group and * denotes significant difference from control ($P<0.05$).

Chapter 6

EFFECTS ON PLASMA POTASSIUM CONCENTRATION

The drugs used in this study interfere with the sympathetic nervous system and some of them, either alone or in combination, have been shown to affect plasma catecholamine levels. Catecholamines have a profound effect on plasma potassium (Bia and DeFronzo, 1981). The plasma potassium level can, in turn, critically affect the incidence and severity of ischaemia-induced arrhythmias (Curtis et al, 1985). Plasma potassium measurements were therefore carried out to investigate any relationship between the effects of the drugs on plasma potassium and their previously described effects on plasma catecholamines and arrhythmias.

6.1. Effects of yohimbine and idazoxan, alone and in combination with desipramine, on the plasma potassium concentration following coronary occlusion

Plasma from blood samples obtained at the end of the 20 min coronary occlusion period from surviving animals in groups treated with the arrhythmogenic doses of yohimbine and idazoxan (0.1 and 0.3 mg/kg respectively), alone and in combination with 0.1 mg/kg desipramine, was assayed for potassium by flame photometry. Similar samples obtained from 14 control rats treated with saline were also analysed. Idazoxan or yohimbine alone at these doses did not significantly alter the plasma potassium concentration. The combinations of 0.1 mg/kg yohimbine and 0.3 mg/kg idazoxan with desipramine slightly reduced the post-occlusion plasma potassium concentration from 5.1 $\pm$ 0.2 mM in the control group to 4.8 $\pm$ 0.1 and 4.9 $\pm$ 0.1 mM respectively. These changes, however, were not statistically significant (Table 6).

6.2. Comparison of potassium concentration determinations by *in vivo* electrodes and *in vitro* analysis by flame photometry

The accuracy of the potassium-selective electrodes was tested by comparing *in vivo* electrode readings with the results obtained from *in vitro* analysis of simultaneously obtained blood samples by flame photometry. The mean plasma potassium concentration values given by electrode measurements and flame photometric analysis were almost identical at 3.92 ± 0.07 and 3.94 ± 0.07 mM respectively (n=15). The mean of the differences between the two sets of measurements was 0 ± 0.05 (n=15) and the electrode readings were not significantly different from flame photometry results when compared by the paired t-test.

6.3. Effects of adrenaline on the plasma potassium concentration

In early experiments, the *in vivo* sensitivity of the ion-selective electrode system to changes in the plasma potassium concentration was tested by recording the responses to intravenous administration of adrenaline, which produces characteristic changes in plasma potassium levels (Coats, 1985). Injection of 250 ng adrenaline produced a triphasic response. There was an initial rapid fall in plasma potassium concentration, which was maximal at around 1 min after injection, followed by a rise above the pre-injection level peaking at around 4 min. This was then followed by a second, slow hypokalaemic phase which was maximal at 8-9 min post-injection. Changes in arterial blood pressure and heart rate in response to adrenaline were very rapid, occurring within seconds of injection and preceding any change in plasma potassium concentration. However, no correlation was observed between the magnitude or direction of the

haemodynamic changes and the subsequent alterations in plasma potassium concentration with adrenaline or any of the other drugs examined. Figure 39 depicts a representative experimental recording illustrating the changes observed in blood pressure, heart rate and plasma potassium concentration in response to the intravenous administration of adrenaline.

6.4. Effects of desipramine on the plasma potassium concentration

Intravenous administration of cumulative doses of desipramine (DMI) had no significant effect on the plasma concentration of potassium in the anaesthetized rat. At no time during the 25 min period following the injection of 0.1, 0.5 and 2.5 mg/kg doses of DMI was the plasma potassium concentration significantly different from the pre-injection value or the corresponding control value obtained from rats injected with saline (Table 7). The blood pressure and heart rate responses to the 3 doses of DMI were similar to those described in Chapter 3.

6.5. Effects of yohimbine, alone and in combination with desipramine, on the plasma potassium concentration

The mean plasma concentration of potassium in the group of rats receiving cumulative doses of yohimbine was slightly lower than controls prior to drug administration. This difference, however, was not statistically significant. Administration of yohimbine at doses of 0.1 and 1.0 mg/kg did not significantly alter the potassium concentration which remained close to the pre-injection value of 3.9 $\pm$ 0.1 mM throughout the 25 min post-injection period (Table 8).

The combined administration of 0.1 mg/kg yohimbine with 0.1 mg/kg DMI produced a rapid reduction in plasma potassium concentration, from a pre-injection value of 4.3 ± 0.1 mM to 4.0 ± 0.1 mM within 1 min of injection. However, this effect was short lasting and the potassium concentration was not significantly different from the pre-injection value by 3 min after injection (Figure 40).

The intravenous administration of yohimbine alone at these dose and the combination of yohimbine and DMI produced similar changes in blood pressure and heart rate to those described in Chapter 3.

6.6. Effects of idazoxan, alone and in combination with desipramine, on the plasma potassium concentration

Intravenous administration of cumulative doses of idazoxan had no significant effect on the plasma potassium concentration. In groups receiving the 0.03 and 0.3 mg/kg doses of idazoxan, the mean plasma potassium concentration remained similar to the pre-injection values of 4.1 ± 0.2 and 4.0 ± 0.3 mM respectively, at all times during the 25 min post-injection period (Table 9).

The mean pre-injection value for plasma potassium concentration was higher at 4.6 ± 0.1 mM in the group receiving the combination of 0.3 mg/kg idazoxan and 0.1 mg/kg DMI than in the control group, which measured 4.2 ± 0.1 mM. This difference between the two groups, however, was not statistically significant. Intravenous administration of this idazoxan/desipramine combination produced a significant reduction in the plasma potassium concentration, from 4.6 ± 0.1 to 4.1 ± 0.1 mM within 2 min of injection. This effect was long lasting and the potassium concentration was still lower than the

pre-injection value at 4.2 ± 0.1 mM 25 min after injection. However, due to the higher pre-injection potassium concentration in this group the plasma potassium concentration was not significantly lower than the corresponding control value at any time following injection (Figure 41).

Idazoxan alone at 0.03 and 0.3 mg/kg and the idazoxan/DMI combination (0.3/0.1 mg/kg) produced significant increases in blood pressure and heart rate similar to those described in Chapter 3. Figure 42 depicts a typical recording showing the changes in blood pressure, heart rate and plasma potassium concentration produced by intravenous administration of the idazoxan/desipramine combination.

6.7. Effects of pretreatment with propranolol on the responses to the combined intravenous administration of idazoxan and desipramine

The combination of idazoxan and DMI has been shown to produce a large increase in plasma noradrenaline concentration and a significant reduction in plasma potassium concentration. These experiments were conducted to investigate whether the hypokalaemic action of this combination was caused by a β-adrenoceptor mediated tissue uptake of potassium, mainly by skeletal muscle.

Intravenous administration of saline 5 min before the idazoxan/DMI combination (0.3/0.1 mg/kg) did not affect the responses to this combination. There were rapid and significant increases in blood pressure and heart rate, accompanied by a significant reduction in the plasma potasium concentration similar to that described in section 6.6. (Figure 43).

When 0.3 mg/kg propranolol was administered intravenously, prior

to the idazoxan/DMI combination, it produced a significant reduction in heart rate from 395 ± 15 to 332 ± 6 beats/min 5 min after injection. This was accompanied by a small but significant increase in the plasma potassium concentration, from a pre-injection value of 4.4 ± 0.1 mM to 4.6 ± 0.1 mM 5 min after injection. Arterial blood pressure was not significantly affected by this dose of propranolol (Figure 43).

Such pretreatment with propranolol did not affect the rapid and significant increase in blood pressure produced by the idazoxan/DMI combination but attenuated the increase in heart rate. Whereas the maximum increase in heart rate was + 85 beats/min achieved at 7 min after injection in the group pretreated with saline, pretreatment with propranolol at 0.3 mg/kg reduced the maximum increase to + 60 beats/min and this occurred much later at 15 min after injection. Propranolol also inhibited the reduction in plasma potassium concentration produced by the idazoxan/DMI combination. Although the plasma potassium concentration was significantly reduced from 4.2 ± 0.3 mM to 3.8 ± 0.4 mM within 2 min of injection in the control group, no such reduction was observed after pretreatment with 0.3 mg/kg propranolol (Figure 43).

Drug	Dose (mg/kg)	n	Plasma potassium concentration (mM)
Saline	-	14	5.1(0.2)
YOH	0.1	12	5.0(0.2)
YOH/DMI	0.1/0.1	13	4.8(0.1)
IDA	0.3	14	5.0(0.1)
IDA/DMI	0.3/0.1	14	4.9(0.1)

Table 6 Plasma potassium concentrations at the end of the 20 min ischaemic period in the control group receiving saline and in groups treated with yohimbine (YOH) and idazoxan (IDA), alone and in combination with desipramine (DMI). Plasma potassium was assayed by flame photometry. All values are means and values in brackets represent s.e.mean.

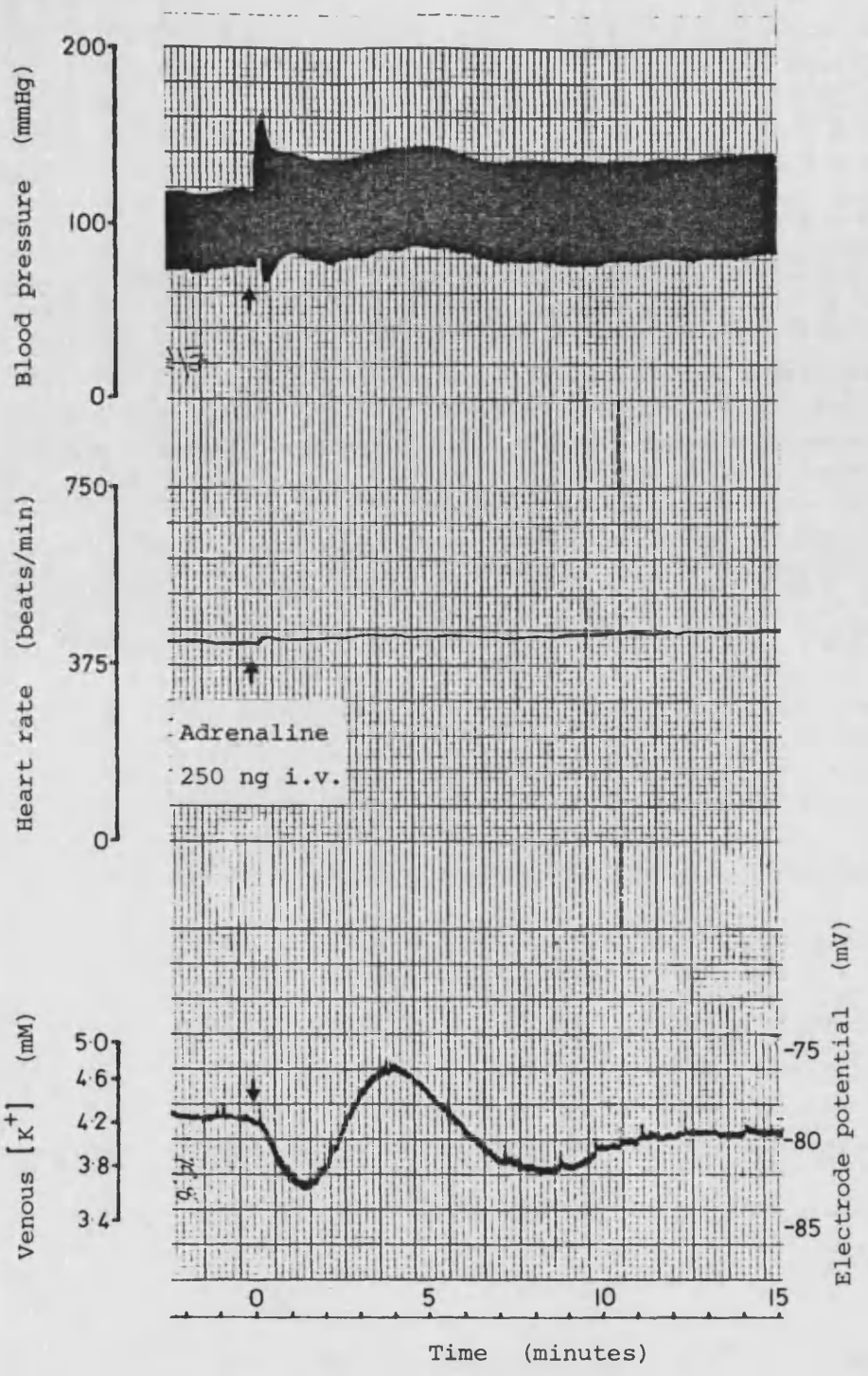


Figure 39 Representative recording illustrating the effects of intravenous administration of 250 ng adrenaline on arterial blood pressure, heart rate and plasma potassium concentration, as monitored by an intravenous potassium-selective electrode.

Drug	Dose (mg/kg)	n	Venous potassium concentration (mM) minutes after administration									
			0	1	2	3	5	7	10	15	20	25
Saline	-	12	4.2(0.1)	4.1(0.1)	4.1(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)
DMI	0.1	3	4.3(0.2)	4.3(0.1)	4.3(0.2)	4.4(0.2)	4.4(0.2)	4.5(0.2)	4.6(0.2)	4.6(0.2)	4.5(0.2)	4.4(0.2)
DMI	0.5	3	4.3(0.1)	4.1(0.1)	4.1(0.1)	4.2(0.1)	4.2(0.1)	4.3(0.2)	4.4(0.2)	4.5(0.2)	4.5(0.2)	4.4(0.2)
DMI	2.5	3	4.3(0.4)	4.3(0.4)	4.4(0.4)	4.5(0.3)	4.3(0.3)	4.3(0.4)	4.3(0.3)	4.4(0.2)	4.5(0.2)	4.4(0.2)

Table 7 Effects of intravenous administration of saline and cumulative doses of desipramine (DMI) on the plasma potassium concentration, as monitored by intravenous potassium-selective electrodes. All values are means and values in brackets represent s.e.mean.

Drug	Dose (mg/kg)	n	Venous potassium concentration (mM) minutes after administration									
			0	1	2	3	5	7	10	15	20	25
Saline	-	12	4.2(0.1)	4.1(0.1)	4.1(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)
YOH	0.1	3	3.9(0.1)	3.9(0.1)	3.9(0.1)	3.9(0.1)	3.8(0.1)	3.8(0.1)	3.8(0.1)	3.9(0.1)	3.9(0.1)	3.8(0)
YOH	1.0	3	3.9(0.1)	3.9(0.1)	4.1(0)	4.1(0)	4.0(0)	3.9(0)	3.8(0)	3.8(0.1)	3.9(0)	3.9(0.1)

Table 8 Effects of intravenous administration of saline and cumulative doses of yohimbine (YOH) on the plasma potassium concentration, as monitored by intravenous potassium-selective electrodes. All values are means and values in brackets represent s.e.mean.

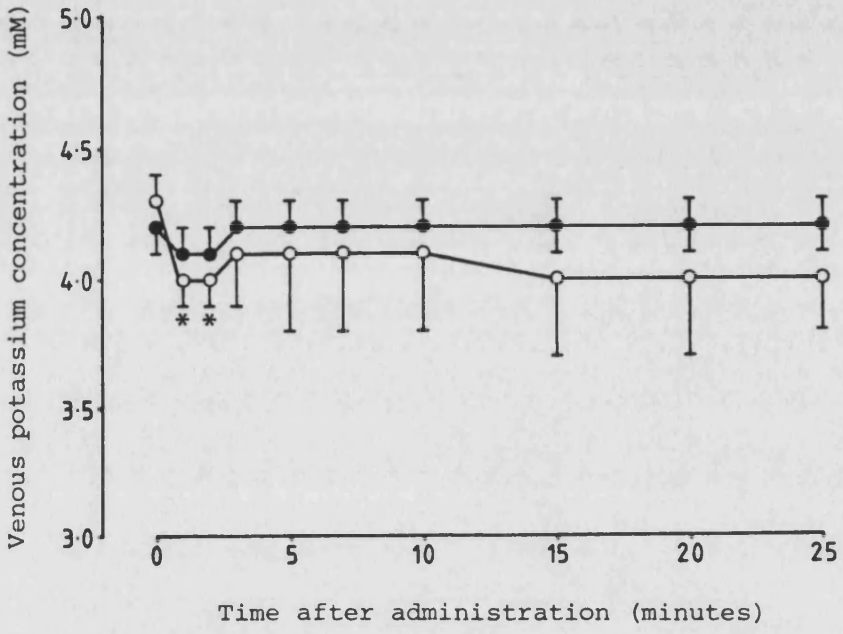


Figure 40 Effects of intravenous administration of saline (●,n=12) and combined yohimbine/desipramine at 0.1/0.1 mg/kg (O,n=3) on the plasma potassium concentration, as monitored by intravenous potassium-selective electrodes. All values are means and vertical lines represent s.e.mean. * denotes significant difference from 0 min value ($P<0.05$).

Drug	Dose (mg/kg)	n	Venous potassium concentration (mM) minutes after administration									
			0	1	2	3	5	7	10	15	20	25
Saline	-	12	4.2(0.1)	4.1(0.1)	4.1(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)	4.2(0.1)
IDA	0.03	3	4.1(0.2)	4.1(0.2)	4.1(0.2)	4.1(0.2)	4.1(0.2)	4.1(0.2)	4.1(0.2)	4.0(0.2)	4.0(0.2)	4.0(0.2)
IDA	0.30	3	4.0(0.3)	3.9(0.2)	3.9(0.2)	4.0(0.2)	4.0(0.2)	3.9(0.2)	4.0(0.2)	4.0(0.2)	4.0(0.2)	3.9(0.2)

Table 9 Effects of intravenous administration of saline and cumulative doses of idazoxan (IDA) on the plasma potassium concentration, as monitored by intravenous potassium-selective electrodes. All values are means and values in brackets represent s.e.mean.

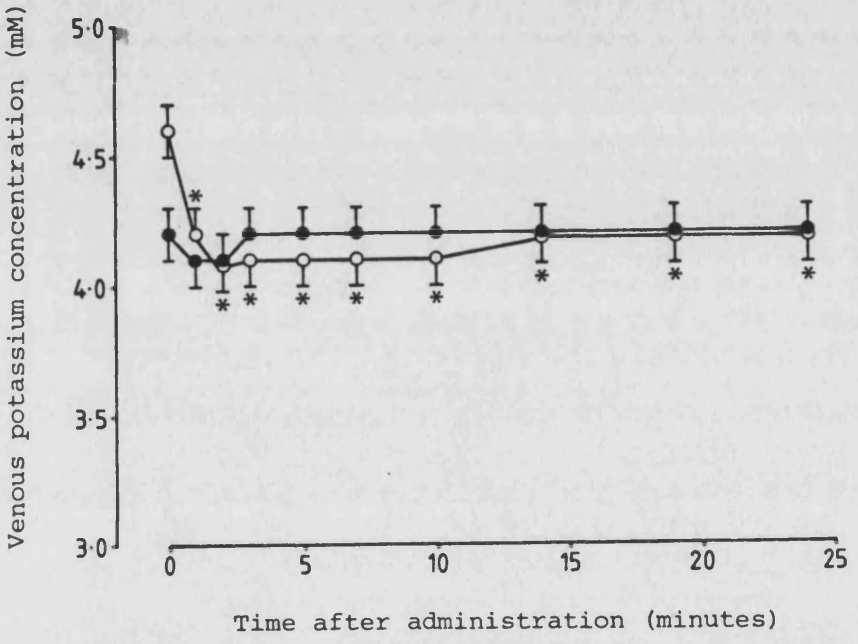


Figure 41 Effects of intravenous administration of saline (●,n=12) and combined idazoxan/desipramine at 0.3/0.1 mg/kg (○,n=3) on the plasma potassium concentration, as monitored by intravenous potassium-selective electrodes. All values are means and vertical lines represent s.e.mean. * denotes significant difference from 0 min value ($P<0.05$).

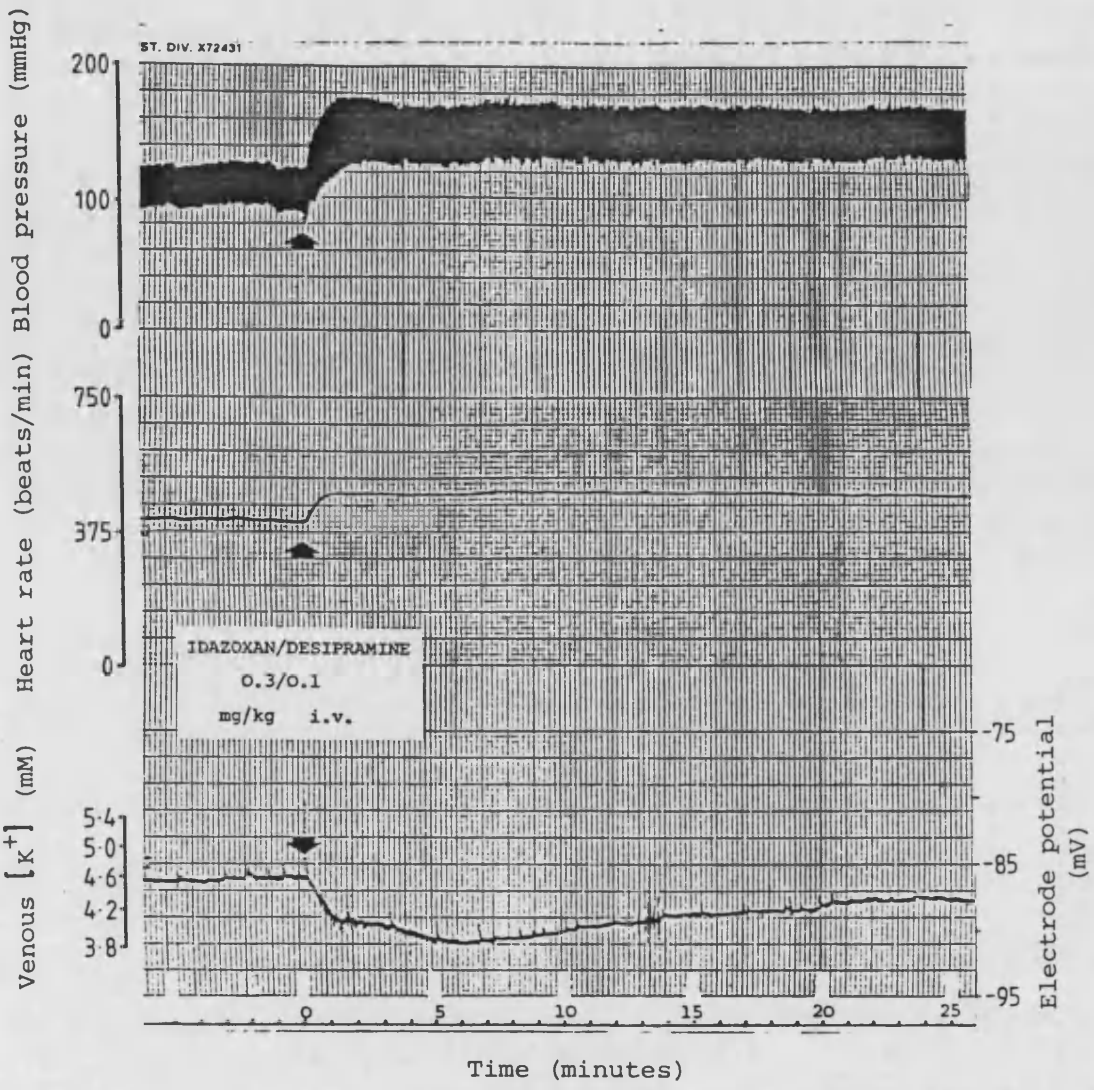
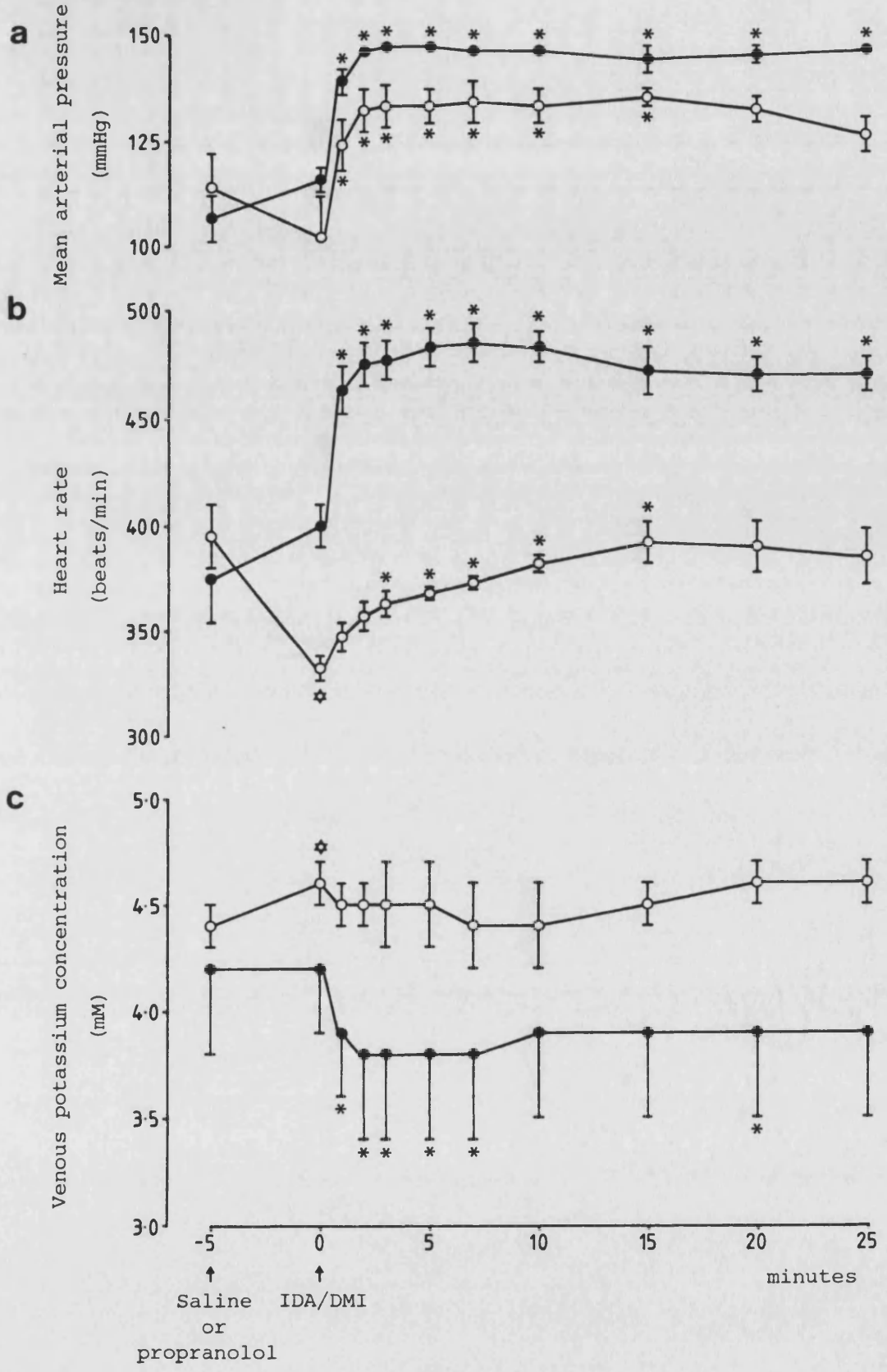


Figure 42 Representative recording illustrating the effects of intravenous administration of combined idazoxan/desipramine at 0.3/0.1 mg/kg on arterial blood pressure, heart rate and plasma potassium concentration, as monitored by an intravenous potassium-selective electrode.

Figure 43 Effects of pretreatment with saline (●,n=3) and 0.3 mg/kg propranolol (O,n=3) on the changes in (a) mean arterial pressure, (b) heart rate, and (c) plasma potassium concentration produced by intravenous administration of combined idazoxan/desipramine (IDA/DMI) at 0.3/0.1 mg/kg. Plasma potassium was monitored by intravenous potassium-selective electrodes. All values are means and vertical lines represent s.e.mean. ✡ denotes significant difference from the -5 min value and * denotes significant difference from the 0 min value ($P<0.05$).


a
Mean arterial pressure
(mmHg)
150
125
100
b
Heart rate
(beats/min)
500
450
400
350
300
c
Venous potassium concentration
(mM)
5·0
4·5
4·0
3·5
3·0
-5
0
5
10
15
20
25
minutes
Saline
or
propranolol
IDA/DMI

Chapter 7

DISCUSSION

7.1. Coronary occlusion-induced arrhythmias in the anaesthetized rat

Throughout the duration of this study arrhythmias were consistently produced by left anterior descending coronary artery occlusion. The type of arrhythmias observed and the time course of their occurence were similar to those previously reported in this model (Clark et al, 1980), although the incidence of ventricular fibrillation was somewhat lower. Indeed, an incidence of ventricular fibrillation varying from 18% to 62% has been reported using the same model (Abrahamsson and Almgren, 1981; Au, Collins, Harvie and Walker, 1979; Clark et al, 1980; Daugherty et al, 1986; Kane et al 1981; Parratt et al, 1981). Such variation is probably the result of the use of different strains and the difficulty in diagnosing ventricular fibrillation in the rat which can spontaneously revert to sinus rhythm (Marshall et al, 1981). In this study, strict diagnostic criteria, such as chaotic electrical activity and a simultaneous reduction in blood pressure towards zero with no pulse, were implemented resulting in a consistent incidence of ventricular fibrillation in five separate control groups. No significant seasonal variation was observed in the incidence and severity of coronary occlusion-induced arrhythmias, contrasting the findings of Abrahamsson and Almgren (1981).

The electrocardiographic changes induced by coronary occlusion in this study, such as the rapid and transient increase in R wave amplitude, the gradual ST segment elevation and the late development of Q waves were also consistent with previous reports (Clark et al, 1980; Kane et al, 1981) and were used as indicators of successful occlusion, which was later confirmed by dye injection. Although no quantitative assessment was made, successful coronary occlusion

consistently produced visually distinct ischaemic zones comprising majority of the left ventricular wall.

In larger animals such as the dog early arrhythmias can be divided into two distinct phases, termed 1a and 1b, which may have different electrophysiological mechanisms (Russell et al, 1984). A similar pattern of early arrhythmias has also been suggested to occur in the anaesthetized rat, with the first phase peaking 5-7 min after coronary occlusion, followed by an abrupt reduction in ectopic activity and then the peak of the second phase at 9-11 min (Parratt et al, 1981; Kane et al, 1981). However the earlier study of Clark et al (1980) showed no evidence for such a division in early ectopic activity. A biphasic pattern of early arrhythmias was not observed with any of the control of drug treatment groups in the present study.

7.2. Effects of coronary occlusion on blood pressure and heart rate

Coronary occlusion produced a significant reduction in blood pressure, which would normally be expected to produce a reflex increase in heart rate. There was, however, no change in heart rate following coronary occlusion, suggesting an impairment of the baroreceptor reflex mechanisms during early myocardial ischaemia. Similar observations have been made in the anaesthetized rat by other workers (Clark et al, 1980; Abrahamsson et al, 1985). In the conscious rat model, however, the coronary occlusion induced fall in blood pressure was accompanied by an increase in heart rate (Johnston, Macleod and Walker, 1981) suggesting that the blunting of the baroreceptor reflex was probably a consequence of anaesthesia.

7.3. Effects of desipramine on blood pressure and heart rate

In this study, desipramine (DMI) produced significant increases in blood pressure and heart rate at doses of 0.1 and 0.5 mg/kg but the increases were smaller with the latter dose. The pressor and positive chronotropic effects observed with DMI are consistent with neuronal uptake blockade elevating the concentration of noradrenaline available for adrenoceptor stimulation in the synapic cleft of sympathetic nerve terminals in the heart and vasculature. However the smaller responses observed with the higher dose suggest that additional actions of the drug may be involved at high doses. Indeed, Graham et al (1980) observed only a transient increase in blood pressure with no effect on heart rate with 1.0 mg/kg DMI in the rat, and intravenous administration of 2.5 mg/kg in this study reversed the effects observed with the lower doses and produced significant reductions in both parameters.

DMI, like other tricyclic antidepressant drugs, has a wide spectrum of pharmacological activity in addition to neuronal uptake blockade (Bowman and Rand, 1980). It has α-adrenoceptor antagonistic properties and can inhibit various ionic channels in the cell membrane at high doses. The relative potency of DMI in blocking noradrenaline uptake is, however, much greater than its α-adrenoceptor blocking action. The effects of DMI on blood pressure and heart rate in this study suggest that at the lowest dose neuronal uptake blockade may be the dominant effect, producing increases in blood pressure and heart rate. With increasing dose, however, other effects also seem to become important, and the reductions in both haemodynamic parameters observed with 2.5 mg/kg DMI may have resulted from postsynatpic α-adrenoceptor antagonism and direct myocardial

depression overcoming the effects of neuronal uptake blockade.

7.4. Effects of desipramine on neuronal uptake

The degree of neuronal uptake blockade produced by the 0.1 and 0.5 mg/kg doses of DMI was assessed using the indirect sympathomimetic drug tyramine. Tyramine produces its effect by being taken up into sympathetic nerve terminals by the neuronal uptake process and releasing noradrenaline by displacing the transmitter from vesicular binding sites. The actions of tyramine can therefore be inhibited by neuronal uptake blockers.

Treatment of rats with DMI shifted the dose-response curves of tyramine to the right. With regard to the pressor effects of tyramine, mediated mainly by noradrenaline release from vasomotor nerve terminals at the adventitial-medial border of arterial walls, the rightward shift of the dose-response curve was greater after 0.5 mg/kg DMI than it was after 0.1 mg/kg DMI, suggesting a greater degree of neuronal uptake blockade with the higher dose. However, the two doses of DMI inhibited the positive chronotropic effects of tyramine to a similar extent. Assuming the positive chronotropic effect of tyramine was mainly mediated by noradrenaline release from cardiac sympathetic nerve terminals, these results suggest that DMI inhibits neuronal uptake within the heart to a similar extent at both 0.1 mg/kg and 0.5 mg/kg doses. Therefore the use of doses greater than 0.1 mg/kg may not produce a greater inhibition of noradrenaline uptake by cardiac sympathetic nerves, but may complicate the intepretation of results by introducing the additional pharmacological actions of DMI that are evident with high doses of the drug.

7.5. Effects of yohimbine and idazoxan on blood pressure and heart rate

The two α_2-adrenoceptor antagonists used in this study produced differing effects on blood pressure and heart rate. Yohimbine had no lasting effect on these parameters at 0.1 mg/kg but produced a sustained reduction in blood pressure and a transient fall in heart rate at 1.0 mg/kg. In contrast, idazoxan produced increases in blood pressure and heart rate at both doses used (0.03 and 0.3 mg/kg), with larger responses observed with the higher dose.

The contrasting haemodynamic effects of yohimbine and idazoxan probably stem from the differences in their potency and selectivity. Doxey, Roach and Smith (1983) have shown idazoxan (RX 781094) to be more potent and more selective than yohimbine as an α_2-adrenoceptor antagonist, with a high degree of specificity. The pressor and positive chronotropic effects of idazoxan can therefore be explained by a substantial blockade of the α_2-adrenoceptors mediating the presynaptic inhibition of noradrenaline release. The pressor effects of idazoxan may also be partly mediated by a partial agonist effect on postsynaptic α_1-adrenoceptors (Ramage and Tomlinson, 1985). Yohimbine, being much less selective than idazoxan, also antagonizes postsynaptic α-adrenoceptors and this may be responsible for the reduction in blood pressure observed with this drug. High doses of yohimbine also produce a reduction in heart rate, which has been suggested to result from an increased vagal tone in the cat (Ramage and Tomlinson, 1985). However, significant reductions in heart rate with comparable doses of yohimbine were observed even after bilateral vagotomy in the anaesthetized rat (Doxey *et al*, 1983) and dog (Paciorek and Shepperson, 1985) indicating a direct effect on the

heart. Indeed, Docherty and McGrath (1979) found in the pithed rat that 1.0 mg/kg yohimbine, in addition to causing a reduction in basal heart rate, also inhibited the tachycardia induced by intravenous noradrenaline, suggesting a postsynaptic effect.

Following intravenous administration, both yohimbine and idazoxan have been shown to produce increases in preganglionic sympathetic nerve activity in the anaesthetized cat, accompanied by elevations in blood pressure and heart rate at low doses (0.03-0.3 mg/kg), suggesting that the excitatory effects of α_2-adrenoceptor antagonism may be centrally mediated (Ramage and Tomlinson, 1985). However, α_2-adrenoceptor antagonism also potentiated the tachycardia evoked by cardiac sympathetic nerve stimulation in the anaesthetized dog (Paciorek and Shepperson, 1985) and pithed rat (Docherty and McGrath, 1979), which indicates a peripheral effect. Indeed, Brown and Harland (1984) have suggested that the effects idazoxan were mainly mediated by peripheral α_2-adrenoceptor antagonism as i.v. idazoxan produced a greater increase in noradrenaline release than i.c.v. administration in the anaesthetized rat.

Therefore, it appears that α_2-adrenoceptor antagonists can enhance sympathetic activity by both central and peripheral α_2-adrenoceptor blockade although after intravenous administration the latter action probably predominates. Such enhanced sympathetic activity leads to increases in blood pressure and heart rate with highly selective antagonists like idazoxan, but with less selective drugs such as yohimbine postsynaptic adrenoceptor antagonism and other non-specific effects may lead to reductions in these parameters, especially at high doses.

7.6. Effects of combined α_2-adrenoceptor antagonism and neuronal uptake blockade on blood pressure and heart rate

Concomitant administration of 0.1 mg/kg DMI, a dose which was shown to produce substantial neuronal uptake blockade with little other action, as judged by its haemodynamic effects, along with α_2-adrenoceptor antagonists yohimbine and idazoxan produced very large increases in blood pressure and heart rate. These increases were substantially greater than those produced by DMI alone at that dose, especially when DMI was given with the more potent α_2-adrenoceptor antagonist idazoxan. These results are consistent with neuronal uptake blockade and antagonism of α_2-adrenoceptor mediated presynaptic inhibition (and possibly a centrally mediated increase in sympathetic output) acting synergistically to produce large increases in noradrenaline levels in the synaptic cleft, leading to enhanced stimulation of postsynaptic α- and β-adrenoceptors. Yamaguchi *et al* (1977) also observed that combined administration of DMI and the non-selective α-adrenoceptor antagonist phenoxybenzamine produced increases in heart rate greater than those produced by DMI alone.

It was interesting to note that when given with 1.0 mg/kg yohimbine, DMI still produced increases in blood pressure and heart rate greater than those observed with DMI alone, despite the reductions in blood pressure and heart rate produced by yohimbine at that dose. This suggests that the increased sympathetic activation produced by the combination was sufficient to nullify the postsynaptic effects of yohimbine at that dose.

7.7. The antiarrhythmic effects of desipramine

DMI has been found to have a dose related antiarrhythmic effect

in vivo in this study, supporting the results of Daugherty *et al* (1986) with the *in vitro* rat heart. It appears doubtful, however, that this effect was related to the blockade of neuronal uptake of noradrenaline by this agent. Results with tyramine have shown that in the non-ischaemic heart 0.1 mg/kg DMI produces maximal neuronal uptake blockade. The haemodynamic observations also suggest that there is little additional pharmacological effect with this dose of DMI. 0.1 mg/kg DMI, however, had no effect on ischaemia-induced arrhythmias whereas the two higher doses, whose haemodynamic actions suggest that they may produce additional pharmacological effects, provided significant protection against ventricular tachycardia and fibrillation and abolished mortality.

There are several mechanisms, unrelated to neuronal uptake blockade, by which DMI can produce an antiarrhythmic effect. The electrophysiological studies of Tamargo, Rodriguez and Garcia De Jalon (1979), using isolated guinea pig ventricular papillary muscles, showed DMI to reduce the maximum rate of rise of phase 0 of the action potential, which is considered a valid index of the Na^+ inward current, suggesting a class I (local anaesthetic) type antiarrhythmic mechanism. They also demonstrated that DMI could inhibit the Ca^{2+}-mediated action potentials (slow responses) induced by isoprenaline when the fast inward Na^+ current was inactivated by elevated extracellular potassium concentrations. Such an effect could protect against re-entry arrhythmias in the setting of myocardial ischaemia.

More recently, Isenberg and Tamargo (1985) have investigated the electrophysiological effects of imipramine, another tricyclic antidepressant closely related to DMI, on isolated bovine ventricular

myocytes using the voltage clamp technique. Their results showed that, in the absence of Na^+, imipramine depressed the slow inward Ca^{2+} current (I_{Ca}), supporting the earlier finding of Tamargo *et al* (1979) that DMI could inhibit Ca^{2+}-mediated slow responses. In addition to a possible contribution to the antiarrhythmic mechanism of high doses of DMI, such and inhibition of I_{Ca} would explain the reduction in heart rate produced by 2.5 mg/kg DMI in the present study as this current is responsible for depolarization in sinoatrial nodal cells. Isenberg and Tamargo (1985) found that imipramine could also slow the final phase of repolarization and prolong the action potential duration by inhibiting the outward K^+ current - another potentially antiarrhythmic effect.

Schomig *et al* (1984; 1985) have suggested that a calcium-independent carrier mediated efflux of noradrenaline may occur during myocardial ischaemia, which uses the same carrier that is normally responsible for transporting noradrenaline from the synaptic cleft back into the neuron and can thus be suppressed by neuronal uptake blockers such as DMI. Such a local efflux of noradrenaline would be highly arrhythmogenic, especially if it was heterogeneous and confined only to areas of severe flow reduction within the ischaemic zone. If such an efflux did indeed occur during ischaemia, its inhibition could contribute to the antiarrhythmic mechanism of DMI. Recently, Aronstam and Hoss (1985) have shown DMI to inhibit depolarization induced calcium uptake by rat synaptosomes, suggesting that DMI could also directly suppress calcium-dependent release processes such as exocytosis. Inhibition of exocytosis could be protective against arrhythmias by inhibiting myocardial noradrenaline release resulting from reflex sympathetic activation and/or release

induced by elevated extracellular potassium.

Other tricyclic antidepressants have recently been reported to inhibit ventricular fibrillation after coronary occlusion in the anaesthetized cat (Manoach, Netz, Varon and Ben-Ze'ev, 1986). These authors also found a reduction in the size of the ischaemic zone after drug treatment but suggested that the protection against fibrillation and the improved collateral blood supply were independent effects. In the present study the ischaemic zone was not quantified but it is hard to envisage any drug treatment reducing its size in the presence of permanent and complete coronary occlusion, due to the low collateral flow in the rat. In contrast to the rat, the cat has a highly developed collateral circulation (Schaper, 1984) and perfusion of the ischaemic area can be improved by pharmacological agents that increase the perfusion pressure or produce coronary dilation.

The results of the present study have shown DMI to be a highly effective antiarrhythmic agent in the anaesthetized rat during myocardial ishaemia although the mechanism of this effect is not clear. Comparison of the effects of various doses of DMI on the responses to tyramine, on haemodynamic parameters, on plasma catecholamine levels (discussed in section 7.11.) and on ischaemia induced arrhythmias dissociate the neuronal uptake blocking and antiarrhythmic actions of this drug. From available evidence, the most likely mechanism of the antiarrhythmic action of DMI appears to be direct inhibition of membrane conductances to Na^+, Ca^{2+} and possibly K^+. However, there is also some evidence suggesting that DMI may be protective by inhibiting myocardial noradrenaline release during ischaemia.

7.8. Effects of yohimbine and idazoxan on arrhythmias

Yohimbine had opposing effects on arrhythmias at two different doses. At 0.1 mg/kg yohimbine significantly increased the incidences of ventricular fibrillation and mortality whereas at 1.0 mg/kg it produced a significant reduction in all parameters of arrhythmic activity. Idazoxan had no significant effect on serious arrhythmias at the lower dose of 0.03 mg/kg but caused a significant increase in the incidence of ventricular fibrillation and tended to elevate mortality at 0.3 mg/kg. These somewhat contradictory results regarding the effects of α_2-adrenoceptor antagonism on ischaemia-induced arrhythmias can again be explained by the differing specificity and selectivity of the two drugs.

As previously discussed, yohimbine is less selective than idazoxan and has some non-specific actions at high doses. Docherty and McGrath (1979) have reported 0.1 mg/kg to be the largest dose of yohimbine which was still sufficiently selective as a presynaptic α_2-adrenoceptor antagonist, following their studies in the pithed rat. It is interesting to note that this was precisely the dose that had a significant arrhythmogenic effect in this study. At doses higher than this, yohimbine can antagonise postsynaptic adrenoceptors and this may be partly responsible for the antiarrhythmic effects observed with the 1.0 mg/kg dose in this study. In addition to postsynaptic adrenoceptor antagonism, high doses of yohimbine may have direct electrophysiological effects which are potentially antiarrhythmic. At concentrations greater than 10^{-6}M yohimbine has been found to reduce the rate of rise of phase 0 of the action potential (by inhibiting the fast inward Na^+ current) in both the isolated rat ventricular free wall (Northover, 1983) and canine

ventricular myocytes (Briggs and Meier, 1986), indicating a class I (local anaesthetic) antiarrhythmic effect. Indeed the protective effects of yohimbine and other α-adrenoceptor antagonists against ischaemia-induced arrhythmias in the isolated rat heart have previously been attributed to their local anaesthetic properties (Thandroyen et al, 1983; Daugherty et al, 1986).

Idazoxan is a potent and highly selective α_2-adrenoceptor antagonist and there is no published evidence suggesting direct electrophysiological actions for this drug. Indeed the haemodynamic effects of idazoxan and its effects on plasma noradrenaline levels (discussed in section 7.11.) observed in this study are consistent with those expected of a potent presynaptic α_2-adrenoceptor antagonist. The arrhythmogenic effects of idazoxan therefore appear to be mediated by α_2-adrenoceptor blockade although postsynaptic α_1-adrenoceptor stimulation may also contribute as idazoxan has been reported to have partial agonist properties (Ramage and Tomlinson, 1985). The lack of effect of the 0.03 mg/kg dose on serious arrhythmias was probably due to insufficient α_2-adrenoceptor blockade at that dose.

The results of this study support the findings of Forfar et al (1983) who observed an increased incidence of ventricular fibrillation following coronary occlusion after α_2-adrenoceptor blockade (with yohimbine) in the anaesthetized dog. In their study, yohimbine potentiated noradrenaline overflow into the ischaemic effluent during sympathetic stimulation, consistent with presynaptic α_2-adrenoceptor blockade within the ischaemic myocardium. Yohimbine also reduced blood flow to the ischaemic myocardium and, in addition

to increasing the incidence of spontaneous ventricular fibrillation, increased the severity and heterogeneity of conduction abnormalities during ischaemia, presumably through increased local noradrenaline release. These effects, however, were observed with a dose of yohimbine (1.0 mg/kg i.v.) that was found to be antiarrhythmic in this study and the effects of higher or lower doses were not reported. The implication is that the rat heart may be more susceptible to the non-specific and non-selective effects of yohimbine than the dog heart. Docherty and McGrath (1979) have indeed reported 0.1 mg/kg to be the largest dose of yohimbine to block α_2-adrenoceptors without additional effects in the rat, as previously discussed.

The present results with yohimbine and idazoxan show that α_2-adrenoceptor antagonism exacerbates ischaemia-induced arrhythmias in the anaesthetized rat, presumably by enhancing the sympathetic activation of the heart. They also demonstrate, however, that high doses of yohimbine may protect against these arrhythmias, most probably due to a non-specific local anaesthetic action. It appears, therefore, that the effect on arrhythmias of less selective and relatively non-specific α_2-adrenoceptor antagonists like yohimbine may depend on the dose used.

7.9. Effects of concomitant administration of desipramine on the actions of yohimbine and idazoxan

Somewhat surprisingly, when 0.1 mg/kg DMI was given in combination with the doses of yohimbine and idazoxan that had increased the incidence of ventricular fibrillation, the arrhythmogenic effect of α_2-adrenoceptor antagonism was abolished

and the parameters of arrhythmic activity returned to the control level. If the arrhythmogenic effect of α_2-adrenoceptor adrenoceptor antagonism was due to an increased noradrenaline release from cardiac sympathetic nerve terminals, concomitant administration DMI at this dose, which inhibits neuronal uptake and has no significant antiarrhythmic effect on its own, would be expected to further exacerbate arrhythmias by blocking the major removal mechanism of released noradrenaline. Indeed the very large increases in blood pressure and heart rate and the significant elevations in plasma noradrenaline levels produced by the α_2-adrenoceptor antagonist/DMI combinations point towards an increased adrenergic stimulation.

There are several possible explanations for this apparent discrepancy. The first one is that DMI, which is a potent antiarrhythmic agent at doses of 0.5 and 2.5 mg/kg, retains some of its antiarrhythmic actions discussed in section 7.7. at the lower dose of 0.1 mg/kg. Such residual activity may not be enough to inhibit spontaneous arrhythmias following coronary occlusion but may be sufficient to prevent further exacerbation induced by enhanced adrenergic activation following combined neuronal uptake/α_2-adrenoceptor blockade.

The second possibility is that the effects of DMI within the ischaemic myocardium may be different from its effects in the non-ischaemic parts of the heart and other sympathetically innervated tissues. Thus, whereas DMI may enhance the effects of presynaptic α_2-adrenoceptor antagonism by blocking neuronal uptake of noradrenaline in the vasculature and left atrium, thereby causing the large increases in blood pressure and heart rate observed with

combined administration , it may have no such effect within the ischaemic left ventricle. In this respect, the carrier-mediated noradrenaline efflux process proposed to occur in the ischaemic myocardium by Schomig et al (1984; 1985) is of interest. If such an efflux did indeed contribute to noradrenaline release within the ischaemic myocardium after acute coronary occlusion, it would be susceptible to inhibition by DMI. In the absence of other antiarrhythmic effects, low doses of DMI may inhibit this efflux without a significant effect on spontaneous arrhythmias, but such an inhibition may blunt the detremental effects of increased exocytotic noradrenaline release induced by α_2-adrenoceptor antagonism, by limiting the noradrenaline concentration in the synaptic cleft.

It is also possible that DMI may actually prevent the increase in noradrenaline release by exocytosis following α_2-adrenoceptor blockade as this process is calcium-dependent and DMI has been shown to inhibit depolarization induced calcium uptake by rat synaptosomes (Aronstam and Hoss, 1985), as previously discussed. However this seems highly unlikely in view of the effects of α_2-adrenoceptor antagonist/DMI combinations on haemodynamic parameters and plasma noradrenaline levels.

One final possibility worth considering is that the large increases in blood pressure and heart rate produced by the combination treatments may result in a reflex increase in vagal tone and such an increase may counteract the arrhythmogenic effects of increased adrenergic stimulation. Indeed such a mechanism has been proposed for the antiarrhythmic effects of pressor drugs and could be abolished by atropine (Marshall et al, 1981). In the present study, bilateral vagotomy did not affect the protection afforded by the high

dose yohimbine/DMI combination but this was probably a result of the overriding local anaesthetic effects of yohimbine at that dose.

When 0.1 mg/kg DMI was given in combination with 1.0 mg/kg yohimbine, the marked antiarrhythmic effect observed with this dose of yohimbine alone was preserved. This combination produced a significant increase in plasma noradrenaline concentration, presumably as a consequence of an increased noradrenaline concentration in the synaptic cleft. There were also large increases in blood pressure and heart rate suggesting that the elevation in the synpatic levels of noradrenaline had overcome any postsynaptic adrenoceptor antagonism by this dose of yohimbine. The fact that the antiarrhythmic effect of 1.0 mg/kg yohimbine was preserved under these conditions suggests that its main antiarrhythmic mechanism was not postsynaptic adrenoceptor antagonism but was more likely to be non-specific membrane stabilization (local anaesthetic effect). As already discussed, DMI may be protective even at the 0.1 mg/kg dose and may contribute to the reduction in ischaemia induced arrhythmias observed with this combination.

Intravenous administration of 1.0 mg/kg yohimbine in combination with 0.1 mg/kg DMI two minutes after coronary occlusion had no significant effect on the incidence and severity of arrhythmias, in contrast to the antiarrhythmic effects of pre-occlusion administration. This finding confirms that a local effect within the ischaemic myocardium was responsible for the protection observed with the pre-occlusion administration of this combination, as the rat has very poor collateral circulation and, in the presence of a complete coronary occlusion, yohimbine and DMI would not enter the ischaemic zone upon post-occlusion administration. It was interesting to note that, even

in the absence of a local antiarrhythmic effect, the increased sympathetic activation produced by this combination did not significantly worsen the arrhythmias induced by coronary occlusion. Although the plasma concentration of catecholamines was not measured in this group, it was reasonable to assume that a significant general sympathetic activation had occured in view of the large increases in blood pressure and heart rate observed upon administration, similar in magnitude to those observed with this combination when administered prior to coronary occlusion. This finding suggests that an increase in general sympathetic activity leading to systemic catecholamine release may not have a detremental effect on ischaemia induced arrhythmias, in the absence of a local effect within the ischaemic myocardium.

The possibility that the antiarrhythmic effect of the yohimbine/DMI combination (1.0/0.1 mg/kg) was due to a reflex increase in vagal activity, in response to the large increases in blood pressure and heart rate, was tested by performing bilateral vagotomy at various times after coronary occlusion in animals treated with this combination prior to occlusion. Bilateral vagotomy at 3 (corresponding to the period prior to the development of arrhythmias), 7 (at the peak of vulnerability to arrhythmias) and 10 min (at the end of the early period of arrhythmias) had no significant effect on the subsequent arrhythmias, suggesting that an increased vagal tone was unlikely to be the mechanism of the protective effect observed with this yohimbine/DMI combination. A substantial vagal tone was, however, present in these animals after the combined administration of yohimbine and DMI, as bilateral vagotomy produced significant increases in mean arterial pressure and

heart rate in all groups, measuring approximately + 15-30 mmHg and + 10-25 beats/min respectively. These results and the lack of an antiarrhythmic effect when the drugs were given after coronary occlusion confirm that the antiarrhythmic effect observed with the pre-occlusion administration of the 1.0/0.1 mg/kg combination of yohimbine and DMI was the results of a local effect within the ischaemic zone, probably mainly due to the local anaesthetic property of the yohimbine component. However, a reflexly increased vagal tone may be important in mediating the abolition of the arrhythmogenic effects of low dose yohimbine (0.1 mg/kg) and idazoxan (0.3 mg/kg) by the concomitant administration of 0.1 mg/kg DMI, in view of the large increases in blood pressure and heart rate produced by these combinations. It is possible that, in the absence of an overriding local anaesthetic effect such as that observed with the high dose of yohimbine, the removal of the vagal influence in these animals may counteract the protective effect of concomitant administration of DMI and may even further exacerbate arrhythmias. Further experiments are required to clarify this point.

In summary, concomitant administration of 0.1 mg/kg DMI abolished the arrhythmogenic effect of α_2-adrenoceptor antagonism by yohimbine and idazoxan despite producing significant sympathetic activation and elevating plasma noradrenaline levels. As DMI did not protect against arrhythmias when given on its own at that dose, further investigation is required to elucidate the mechanism of its protective effect when given in combination with the arrhythmogenic doses of yohimbine and idazoxan. It is possible that a reflex increase in vagal tone may have been responsible for this protection.

When given with 1.0 mg/kg yohimbine, DMI did not affect the protection against ischaemia-induced arrhythmias afforded by this high dose of yohimbine, which was most probably mediated by a local anaesthetic effect within the ischaemic myocardium.

7.10. Plasma catecholamine levels

Plasma concentrations of noradrenaline and adrenaline were measured 3 minutes after coronary occlusion or sham occlusion. In the anaesthetized rat this corresponds to the period just prior to the development of arrhythmias. The objective was to establish if there was any correlation between the effects of drugs on plasma catecholamine levels and their effects on the incidence and severity of subsequent arrhythmias, in order to delineate any causal relationship. The catecholamine measurements and arrhythmia analyses were performed in separate groups of similarly prepared animals as about 3 ml of blood was required and it was impractical to perform such sampling during arrhythmia experiments due to the small size and low blood volume of the rat.

The plasma concentrations of catecholamines in sham-occluded and control animals were not significantly different and similar to those found by other workers in the anaesthetized rat (Popper, Chiueh and Kopin, 1977). The implication of the similarity in plasma catecholamine levels in sham-occluded and control rats is that coronary occlusion *per se* does not induce an increase in the plasma catecholamine concentration prior to the development of arrhythmias. This does not mean, however, that an increased sympathetic activity does not occur following coronary occlusion as, in the presence of intact neuronal uptake and presynpatic inhibition, an increased

noradrenaline turnover may not necessarily lead to elevated plasma levels (Forfar et al, 1985).

The plasma catecholamine concentrations measured in this study were significantly lower than those reported by Daugherty et al (1986) in the same model, when the blood samples were collected after 30 min of coronary artery occlusion, following the period of ventricular arrhythmias. This suggests that the elevated plasma levels of noradrenaline and adrenaline observed by Daugherty et al (1986) were probably a consequence of the ischaemia induced arrhythmias rather than their cause. Indeed in their study, following acute adrenalectomy or chronic adrenal demedullation, plasma concentrations of noradrenaline and adrenaline were not elevated by 30 min of myocardial ischaemia but these processes did not affect any of the parameters of ventricular arrhythmias.

Therefore, coronary occlusion in the anaesthetized rat did not produce any increase in plasma catecholamine levels after 3 min, prior to the development of arrhythmias. The elevated levels previously observed in the same model at the end of a 30 min coronary occlusion period were probably the result of a reflex release by the adrenal medulla in response to the rapid haemodynamic fluctuations caused by the arrhythmias (Daugherty et al, 1986). These results, taken together, provide evidence that plasma catecholamines are not necessary mediators of the production of arrhythmias following coronary artery occlusion. However, they do not exclude the possibility that catecholamines released locally within the ischaemic myocardium may be important arrhythmogenic mediators.

7.11. Effects of neuronal uptake blockade and α_2-adrenoceptor antagonism on plasma catecholamine levels and their relationship to arrhythmias

DMI did not affect plasma adrenaline but tended to elevate the plasma noradrenaline concentration at 0.1 mg/kg, although the increase was not statistically significant. The lack of a significant increase in plasma levels in the presence of a reduced neuronal uptake was probably the result of presynaptic inhibition of release. Indeed Cousineau et al (1986) have shown in the dog heart that after DMI treatment the interstitial release of noradrenaline was reduced to the same extent as neuronal uptake was diminished and, as a result, the concentration of noradrenaline in the extracellular space was not significantly increased. The lack of an effect on plasma adrenaline was consistent with the finding that the rat adrenal medulla, which is the major source of circulating adrenaline, has no uptake system analogous to that at sympathetic nerve terminals (Wakade and Wakade, 1984).

The higher doses of DMI, which had significant antiarrhythmic effects, had no effect on plasma noradrenaline, possibly because of a further reduction in neuronal noradrenaline release due to the local anaesthetic effects of high doses of DMI. Indeed, these doses produced a reduction in the plasma concentration of adrenaline probably due to such a membrane effect. The release of adrenaline from the adrenal medulla occurs by a calcium-dependent exocytosis similar to that observed in sympathetic nerve terminals (Blaustein, 1979) and can be inhibited by local anaesthetics which block sodium and calcium channels (Hausler and Haefely, 1979). DMI has indeed been shown to inhibit the depolarization-induced uptake of calcium by rat

brain synaptosomes (Aronstam and Hoss, 1985) and could conceivably inhibit adrenaline release from the adrenal medulla by a similar mechanism at high doses. These results confirm that the antiarrhythmic effects of high doses of DMI are not related to the blockade of neuronal uptake of noradrenaline and are more likely to be the consequence of non-specific stabilizing effects on excitable membranes, reducing the conductances to various cations.

Yohimbine did not affect plasma catecholamine levels at either dose. The inability of yohimbine to alter plasma adrenaline levels is not surprising because the rat adrenal medulla does not possess a negative feedback mechanism mediated by α_2-adrenoceptors unlike sympathetic nerve terminals (Sharma et al, 1986). The low dose of yohimbine, which exacerbated ischaemia-induced arrhythmias, did not increase plasma noradrenaline levels. This was probably due to insufficient α_2-adrenoceptor antagonism at that dose to produce an increased overflow into the circulation in the presence of an intact neuronal uptake process, although an increased turnover may have been present. The lack of effect on plasma noradrenaline levels at the higher dose, which significantly inhibited arrhythmias, was possibly due to the local anaesthetic effect of yohimbine counteracting any increased release resulting from α_2-adrenoceptor blockade. These results are consistent with high doses of yohimbine providing protection by a non-specific local anaesthetic effect.

Idazoxan, which is a more potent and more selective α_2-adrenoceptor antagonist than yohimbine, produced a significant increase in the plasma noradrenaline concentration at the dose that exacerbated ischaemia induced arrhythmias. Plasma adrenaline was

again not affected, presumably for the same reason as that for yohimbine, discussed above. Similar observations regarding the effect of intravenous idazoxan on plasma noradrenaline levels have been reported by Brown and Harland (1984) who suggested that the effect was mediated by antagonism of peripheral rather than central α_2-adrenoceptors as i.v. idazoxan produced a greater increase in plasma noradrenaline concentration than i.c.v. administration. In the absence of any local anaesthetic effect, the arrhythmogenic effect observed with this dose of idazoxan was most probably due to the antagonism of presynaptic α_2-adrenoceptors within the ischaemic myocardium leading to increased noradrenaline release. Indeed Forfar *et al* (1983) have shown α_2-adrenoceptor antagonism to increase stimulation induced overflow of noradrenaline from the ischaemic dog myocardium and to significantly increase the incidence of spontaneous ventricular fibrillation.

Combined α_2-adrenoceptor antagonism and neuronal uptake blockade produced highly significant increases in plasma noradrenaline concentration by inhibiting the two most important mechanisms regulating the concentration of noradrenaline in the synaptic clefts of sympathetic nerve terminals. Plasma adrenaline was not affected for reasons previously explained. Significant increases in arterial plasma levels of noradrenaline by combined α_2-adrenoceptor antagonism and neuronal uptake blockade have been reported by other workers (Graham *et al*, 1980; Forfar *et al*, 1985). Forfar *et al* (1985) also showed such an intervention to produce a spontaneous noradrenaline release from both the ischaemic and non-ischaemic zones of the dog heart following coronary occlusion, suggesting that a reflex increase in cardiac sympathetic drive may occur following coronary

occlusion but, under normal circumstances, noradrenaline overflow into the coronary venous effluent may be limited by intact α_2-adrenoceptor mediated negative feedback and neuronal uptake processes. In this study, despite the significant elevations in plasma noradrenaline concentration, combined α_2-adrenoceptor antagonism and neuronal uptake blockade did not exacerbate the arrhythmias induced by coronary occlusion and in fact appeared to have a protective effect, as already discussed. These results suggest that either an elevated plasma noradrenaline concentration is not detremental to the ischaemic heart with regard to arrhythmias, or other pharmacological effects of the agents used to elevate plasma noradrenaline levels and/or reflex changes in neural activity counteract any detremental effects resulting from such a systemic increase in adrenergic stimulation.

Indeed, one of the most pertinent points to emerge from this study is that local effects within the ischaemic myocardium, such as changes in membrane conductances to ions and alterations in local release of noradrenaline, are more important in determining what influence pharmacological agents have on the severity of ischaemia-induced arrhythmias than effects on systemic catecholamine release. Other workers have dismissed circulating catecholamines as important mediators of ischaemia-induced arrhythmias (Daugherty *et al*, 1986). The greater importance of local noradrenaline release within the ischaemic myocardium over circulating catecholamines in determining the severity of ischaemia-induced arrhythmias has been demonstrated by the studies of Martin and Meesman (1985) in the dog, utilizing regional myocardial chemical sympathectomy. In conclusion, although

plasma levels of catecholamines are useful indicators of general sympathetic activity in this model, they do not appear to significantly influence the severity of ischaemia-induced arrhythmias and do not provide a reliable means of predicting the effects of drugs on these arrhythmias.

7.12. Effects of blood pressure and heart rate on arrhythmias

In this study, no correlation was found between blood pressure or heart rate and the severity of ischaemia-induced arrhythmias. Johnston, Macleod and Walker (1983) have reported a similar lack of correlation between these parameters in the conscious rat. In addition, in the anaesthetized dog, Verrier, Thompson and Lown (1974) found that increases in arterial pressure and heart rate induced by stellate ganglion stimulation were not involved in the ability of such stimulation to lower the ventricular fibrillation threshold. These authors suggested that the electrical instability resulting from sympathetic nerve stimulation derived from direct cardiac effects rather than from the accompanying modifications in heart rate and blood pressure. The results of the present study also suggest that blood pressure and heart rate are not important determinants of the severity of arrhythmias following coronary occlusion.

Although changes in blood pressure and heart rate may not have a direct effect on the electrical outcome of myocardial ischaemia, they may have indirect effects by producing reflex changes in vagal tone. Indeed Marshall *et al* (1981) have found a variety of pressor agents, including adrenaline and noradrenaline, to confer protection against coronary occlusion-induced arrhythmias in the anaesthetized rat by increasing the vagal tone, an effect which could be abolished by

prior administration of atropine. Verrier and Hagestad (1985) have also proposed that vagal activation can exert a protective effect on ventricular vulnerability to arrhythmias in the presence of an elevated sympathetic tone. As already discussed, an increased vagal tone may be involved in the protection against ischaemia induced arrhythmias provided by some dome drug interventions that produced large increase in blood pressure and heart rate in this study.

7.13. Plasma potassium measurements

The ion-selective electrode system used for intravascular monitoring of plasma potassium in this study has proved to be an accurate and reliable method, as indicated by the close agreement between electrode readings and the results from flame photometric analysis of simultaneously obtained samples. The sensitivity of the electrodes to changes in plasma potassium concentration was demonstrated by the triphasic responses to the intravenous administration of adrenaline that are characteristic of the rat (Coats, 1985). The resting plasma potassium concentration values measured by these electrodes were also in agreement with previously reported values in the anaesthetized rat (Coats, 1985).

It was interesting to note that the plasma potassium concentrations (measured by flame photometry) were higher in samples obtained from thoracotomized, coronary artery occluded rats at the end of the 20 min ischaemic period than the basal concentrations measured with the potassium-selective electrodes in closed chest anaesthetized animals. These results suggest that the processes of thoracotomy and/or coronary occlusion produced elevations in plasma

potassium concentration. It was not clear from this study whether the increases were the result of myocardial ischaemia or the surgical procedure, as acute surgery itself has been shown to produce significant elevations in plasma potassium concentration (Curtis, Macleod and Walker, 1985).

7.14. Effects of neuronal uptake blockade and α_2-adrenoceptor antagonism on plasma potassium levels and their relationship to arrhythmias

The investigation of the effects of α_2-adrenoceptor antagonism and neuronal uptake blockade on plasma potassium in closed chest anaesthetized rats using potassium-selective electrodes revealed that drug interventions that had been shown to elevate plasma noradrenaline levels in coronary occluded animals produced significant reductions in plasma potassium concentration. Accordingly, yohimbine and DMI alone did not affect plasma potassium but yohimbine/DMI and idazoxan/DMI combinations produced significant reductions. The only exception was idazoxan which did not significantly reduce the plasma potassium concentration despite producing a significant increase in plasma noradrenaline levels. One possible explanation for this observation is that the partial agonist property of idazoxan at α_1-adrenoceptors may have potentiated the α-adrenoceptor mediated hyperkalaemic effects of circulating noradrenaline, thereby cancelling the normally dominant β_2-adrenoceptor mediated hypokalaemic effects via increased uptake by skeletal muscle cells.

The mechanism of the hypokalaemic effect of combined α_2-adrenoceptor antagonism and neuronal uptake blockade was investigated

by studying the effects of pretreatment with propranolol on the responses to intravenous administration of the idazoxan/DMI combination. Propranolol, at a dose previously reported to block the hypokalaemic action of adrenaline in the rat (Coats, 1985), greatly attenuated the reduction in plasma potassium concentration produced by the idazoxan/DMI combination, confirming the involvement of β-adrenoceptors (probably β_2-adrenoceptors in skeletal muscle) in mediating the hypokalaemia. The positive chronotropic effect of this combination was also inhibited as a result of cardiac β-adrenoceptor blockade. These results support the proposition that the effects of combined α_2-adrenoceptor antagonism and neuronal uptake blockade on plasma potassium were mediated by increased noradrenaline release and subsequent β-adrenoceptor stimulation, resulting in increased tissue uptake of potassium.

Plasma potassium concentration is an important determinant of the severity of ischaemia induced arrhythmias, as discussed in Chapter 1, section 1.11.. Therefore, interventions that reduce the plasma concentration of potassium would be expected to exacerbate such arrhythmias. As already discussed, however, concomitant administration of DMI abolished the arrhythmogenic effects of α_2-adrenoceptor antagonism by an unknown mechanism, despite producing a significant increase in plasma noradrenaline concentration and a significant reduction in plasma potassium concentration. Although this study does not provide evidence regarding the nature of the protection afforded by concomitant administration of DMI, it must be a potent effect to provide protection against arrhythmias in the presence of increased adrenergic activation and reduced plasma

potassium concentration.

Another point of interest is that when the potassium concentration was measured in plasma samples obtained at the end of the ischaemic period in coronary artery occluded rats, no significant difference was observed between control animals and those treated with α_2-adrenoceptor antagonist/DMI combinations prior to occlusion. The implication of these results is that increases in plasma potassium concentration produced by the thoracotomy/coronary occlusion process overshadow the relatively small reductions produced by the combined drug treatment. Under these circumstances, α_2-adrenoceptor antagonist/DMI combinations would not be expected to significantly alter arrhythmias via their effects on plasma potassium concentration. Further studies are required, using the intravascular potassium-selective electrodes, to delineate the effects of thoracotomy/coronary occlusion on plasma potassium and the effects of the drugs on plasma potassium in animals subjected to these processes.

7.15. Arrhythmias in vitamin E deficient rats

This study was performed in support of another investigation into the effects of vitamin E deficiency on lipid peroxidation induced by ischaemia in the isolated rat heart (Zakaria, 1985). Vitamin E is the major lipid soluble antioxidant in plasma (Burton, Joyce and Ingold, 1982) and would be expected to protect against free radical oxidation (Dormandy, 1978). In this study, there was no significant difference in the incidence of serious arrhythmias such as ventricular tachycardia and fibrillation between control rats and those fed on a vitamin E deficient diet for 8 weeks. There was

evidence, however, of an increased sympathetic tone following occlusion and the number of premature ventricular contractions was reduced.

The lack of effect on serious arrhythmias suggests that there was not a significant difference in ischaemia-induced membrane damage in the two groups. Indeed Zakaria (1985) found no evidence of increased lipid peroxidation in the vitamin E deficient group and suggested that the 45% reduction in plasma vitamin E concentration produced by 8 weeks of depleted diet was insufficient. Other workers have reported a much more significant reduction (96%) in plasma levels by keeping rats on a vitamin E deficient diet for 7 months (Falanga, Doni, Delaini, Vitti, Imberti, Donati and De Gaetano, 1983). Another equally valid explanation is that free radical oxidation may not play a major role in membrane damage during myocardial ischaemia due to the low availability of oxygen (see Chapter 1, section 1.13.(4)).

7.16. Conclusions

Desipramine (DMI) had a dose related antiarrhythmic effect, probably due to its ability to inhibit membrane conductances to sodium and calcium ions. Antagonism of α_2-adrenoceptors with yohimbine and idazoxan exacerbated ischaemia-induced arrhythmias, presumably as a result of enhanced local noradrenaline release. High doses of yohimbine, however, could protect against these arrhythmias by virtue of the ability of yohimbine to inhibit the fast sodium channel at high concentrations. Paradoxically, concomitant administration of DMI, at a dose that did not affect arrhythmias on

its own but appeared to inhibit neuronal uptake, abolished the arrhythmogenic effects of α_2-adrenoceptor antagonism. The mechanism of this protection could not be deduced from the present data.

Measurement of plasma catecholamine and potassium concentrations revealed no direct relationship between the effects of drugs on these parameters and their effects on ischaemia-induced arrhythmias. There was also no significant correlation between haemodynamic parameters (blood pressure and heart rate) and the severity of ischaemia-induced arrhythmias in any of the groups.

These results show that DMI is a potent antiarrhythmic agent in the rat, probably by a mechanism unrelated to neuronal uptake blockade, while antagonism of inhibitory presynaptic α_2-adrenoceptors exacerbates arrhythmias. Plasma catecholamines, blood pressure and heart rate do not appear to be major determinants of the severity of ischaemia-induced arrhythmias in this model and, in this respect, local effects of the drugs within the ischaemic zone are of greater importance. Further studies are required regarding the importance of drug induced changes in plasma potassium in determining the electrical outcome of myocardial ischaemia.

7.17. Suggestions for future studies

There are several studies which can be carried out in order to ascertain the importance and possible mechanisms of present observations:

1. Investigation of the effects of post-coronary occlusion administration of DMI and the α_2-adrenoceptor antagonists to determine if their respective antiarrhythmic and arrhythmogenic effects were locally mediated.

2. Investigation of the effects of vagotomy or pretreatment with atropine on the responses to lowdose yohimbine/DMI and idazoxan/DMI combinations to determine if, in the absence of a local anaesthetic effect, the observed protection was due to an increased vagal output.

3. Investigation of the effects of the drug interventions used in this study on myocardial noradrenaline levels following coronary occlusion, possibly just prior to the development of arrhythmias (i.e. at 3 min), to determine if there are any drug induced changes in local release and if any such changes correlate with the effects of the drugs on arrhythmias (reported in this study).

4. Investigation of the effects of α_2-adrenoceptor antagonists on arrhythmias in rats pretreated with α-methyl-meta-tyrosine, which has been reported to reduce myocardial noradrenaline levels in rats to <10% of control with minimal effects on other biochemical and haemodynamic parameters (Abrahamsson et al, 1985), to determine if the arrhythmogenic effects of these agents were mediated by increased local release of noradrenaline.

5. Investigation of the electrophysiological effects of DMI on rat cardiac tissue. The studies reported so far have been on guinea-pig and bovine tissues and cells (Tamargo et al, 1979; Isenberg and Tamargo, 1985).

6. Investigation of the effects of the thoracotomy/coronary artery occlusion process on plasma potassium concentration, using the ion-selective electrode system developed in this study, and studying the effects of drugs on any such changes and their relationship to subsequent arrhythmias.

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ADDENDUM

I. There is no significant difference in any of the parameters of arrhythmic activity between the two groups receiving YOH/DMI before or after coronary occlusion (section 4.5., figure 27), although there is a tendency towards more severe arrhythmias in the latter group.

II. Although bilateral vagotomy did not appear to affect the severity of subsequent arrhythmias (section 4.6.) the number of experiments in each group was too small to make the results conclusive.

III. The results of this study show no correlation between blood pressure or heart rate and the arrhythmia score (section 4.9.). There was also no significant difference in these parameters measured before or 5 min after coronary occlusion between animals that exhibited VF and those that did not, within the control group (n=73):

		VF (n=21)	NO VF (n=52)	
0 min	BP	102(4)	98(3)	mmHg
	HR	474(7)	472(5)	beats/min
5 min	BP	77(4)	72(2)	mmHg
	HR	465(9)	465(6)	beats/min

Values in brackets indicate s.e.m.

IV. As plasma catecholamine concentrations and arrhythmias were analysed in separate animals the results suggest but do not prove that there is no relationship between the two parameters.